# ANNUAL REPORT
# OF
# PROGRAM ACTIVITIES

## NATIONAL INSTITUTES OF HEALTH

## 1967 - 1968

## NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

## VOLUME I

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NATIONAL INSTITUTES OF NEUROLOGICAL DISEASES AND BLINDNESS

ANNUAL REPORT of program activities

July 1, 1967 through June 30, 1968

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ANNUAL REPORT

July 1, 1967 through June 30, 1968

National Institute of Neurological Diseases and Blindness
National Institutes of Health

# The Director's Report

This past year's continued vigor and productivity have characterized the Institute's program throughout the research spectrum. Among the most dramatic developments highlighting the area of clinical investigation have been those relating to the role of unusual viral-like agents in degenerative disorders of the nervous system. These findings have wide implications for other chronic neurological disorders.

In kuru, the role of a scrapie-like agent has now been well established. This year, a similar agent was discovered in another chronic neurological disease, spongiform encephalopathy. The role of a viral agent in subacute sclerosing panencephalitis is also virtually proven. There is now evidence that this disease is much more prevalent than previously recognized, and there are indications that an atypical or incomplete form of measles virus is responsible. These studies require long periods of time because of the long latent period of many of these agents. Vast resources for animal holdings and laboratory facilities are necessary as well as unusual dedication and patience on the part of the scientists involved. Much of the work has been accomplished within the Institute's own laboratories in the Bethesda area, and provisions are being made for additional resources for this program. This exciting field is now being recognized as an important area of interest.

Advances stemming from a broadening understanding of the biochemistry of neurological diseases is also dramatic. In practical terms, the development of L-Dopa for the relief of parkinsonism could have a most significant impact on this major neurological disability. Now under clinical investigation by Institute-supported scientists, this drug represents a broad national and international research program on the basic chemistry of the nervous system.

The Institute's therapy evaluation program is continually improving in strength and sophistication. In addition to the evaluation of L-Dopa and new epilepsy drugs, studies of the surgical treatment of intracranial aneurysms and of the efficacy of ACTH in treating multiple sclerosis are nearing completion. New projects for evaluating antihypertensive agents in the prevention of stroke and chemotherapy in brain tumors also are in progress.

Because of the continuing concern about the hazards of oxygen use in premature infants, the Institute, in collaboration with the National Institute of Child Health and Human Development, is developing a program to provide more precise knowledge of the tolerable limits of oxygen therapy. NINDB's previous study of the hazard of blindness from use of oxygen did not provide definitive answers regarding the level of oxygen which can safely be administered.

Effective therapy demands a balance between avoiding the chance of blindness while treating the respiratory distress syndrome. The use of oxygen for treating this syndrome in premature infants has strong justification as a life-saving measure. There are indications, however, that recently some increase in the overall frequency of retrolental fibroplasia has developed.

Certain research areas have been recognized by the Institute staff and consultants as areas of particular need and opportunity. Thus, advances in our knowledge of the form and function of the nervous system parallel the evolution of highly sophisticated electronic and computer devices and techniques. This research indicates that the duplication and supplementation of neural functions is a reasonable goal. Particularly challenging is the possibility of bypassing defective portions of the nervous system and replacing them with external devices, or of using direct coupling of these devices to the nervous system to increase normal function. Fundamental to these advances are three requirements: a precise understanding of the nature of neural activity; instrumentation to duplicate or supplement such activity; and a method whereby an external device can be coupled directly to the human or animal nervous system. Of these three, coupling devices probably are the most crucial and will be the most difficult to attain. The Institute's new Laboratory of Neural Control is seeking answers to these problems.

The development of precise diagnostic measures to visualize cerebral circulation is of the highest importance in the field of clinical neurology. Two types of information are needed for reliable diagnosis: a picture of the structure of the blood vessels supplying the brain; and a qualitative estimate of regional blood flow. Rapid advances in this methodology have been made in this field in recent years. These involve the use of radio-opaque dyes which outline the blood vessels, and the use of tracer elements whose travels through the central nervous system can be monitored by scanning devices. The former test still requires intra-arterial injection of the radio-opaque dyes which is not without hazard, and radioactive tracers do not provide a clear picture of the actual course of the vessels. Refinement of these techniques or the development of others is an immediate practical problem.

Although there have been continued advances in controlling convulsive disorders by the use of anticonvulsive drugs as well as progress in understanding the fundamental mechanisms of these disorders, control of seizures for many patients still has not been found.

Many different disorders are involved in the production of epilepsy. A more precise classification of these disorders is needed as a step toward rationally and systematically evaluating the new anticonvulsant agents. Each year, several new anticonvulsant drugs are developed, usually as a byproduct in the development of tranquilizing or antidepressant agents. There is urgent need for a national study to test these new anticonvulsants, but the Institute has had difficulty in developing such a collaborative study.

The NINDB research program in convulsive disorders is directed toward three main goals: (1) to obtain more specific knowledge on the physiological mechanisms that lead to an epileptic attack; (2) to gain knowledge and understanding of the natural history of the development of seizures and

seizure patterns in the patient; and (3) to develop more effective anti-epileptic drugs and more specific and accurate knowledge of mode of action of drugs for the control of epilepsy.

The problem of senile degenerative disease is still largely overlooked. Within the large State neuropsychiatric institutions, a large proportion of the older patients suffer from degeneration of the central nervous system whose nature and cause is little understood. Studies of amyotrophic lateral sclerosis and of the peculiar parkinsonism-dementia complex seen with great frequency on the island of Guam may have important implications in this field and should be promoted with vigor.

To study this disease, the Institute has maintained a research laboratory on the island of Guam for the past 10 years. To date efforts to determine an environmental factor responsible for this high incidence have not been successful. Pathological examination reveals that in these patients, and in patients with a closely related form of amyotrophic lateral sclerosis, certain specific pathological changes of the brain are unusually prominent. Epidemiological and pathological studies now in progress need to be intensified to determine the significance of these changes with the hope that the causes of these diseases can be finally determined.

In the area of chronic neurological disorders of childhood, the Institute's major effort has been in the Collaborative Perinatal Project. The obstetrical phase with 60,000 pregnancies has been completed, and the oldest children born in the study are now approaching 8 years of age. All of the data on the obstetric aspects are now coded and available on computer tapes, including complete data on 14,000 children through the age of 4 years.

As more information becomes available, the value of the Perinatal Project becomes increasingly evident. This past year, a statistical review committee has been evaluating the data and their report is in its final draft. On the basis of this evaluation, it is hoped that a cost reduction can be achieved by eliminating certain examinations or by using sub-samples of the total study population. Under development is a mechanism for distributing data systematically to the many individuals who would find it useful. Focused studies are being designed to answer specific questions raised as the data is analyzed.

At the Laboratory of Perinatal Physiology in Puerto Rico investigations are providing precise information on the mechanism of action and impact of various types of perinatal causes of cerebral palsy and mental retardation. The Laboratory now has over 1,000 monkeys and the free-ranging colony provides an opportunity to study deviations of the animals' complex social behavior produced by social and physical manipulation.

More precise knowledge about the evaluation and management of the neurologically deviant child is a problem of increasing concern. A series of task force studies are providing information about "minimal brain dysfunction." Task Force I has published a definition of the problem, Task Force II is in the final stages of preparing material on the resources and services required by children with minimal brain dysfunction, and the report from Task Force III

on research needs should be available this year. The Institute also is providing support for a Departmental committee on dyslexia.

One of the major targeted research areas of the Institute is cerebrovascular disease. In addition to an extensive program of research projects in this area, 18 cerebrovascular research centers are now being supported in major population centers of the country. Each center is concentrating on a major approach to the disease problem, such as epidemiology, natural history, the dynamics of cerebral blood flow, the biochemistry of blood constituents, the effect of anticoagulant drugs, cerebral cellular metabolism, and aphasia.

Cooperative studies are comparing the incidence and character of stroke in foreign countries with that in the United States. In Japan, studies have demonstrated that contrary to previous opinion, cerebral hemorrhage is not the major cause of stroke in Japan. As in the United States, cerebral thrombosis has been found to be the most frequent form of the disorder. Recently reported studies in India suggest that there are strikingly different characteristics of disease in the cerebral blood vessels of patients dying with stroke in that country. These studies should provide a clearer understanding of the role of environmental factors in cerebrovascular disease.

A prominent feature of a number of mysterious degenerative neurological disorders is a scar-like sclerosing process, particularly involving scar tissue in the fiber tracts of the brain and spinal cord. Multiple sclerosis, the most common disease of this group, is a disease of temperate climates. A worldwide epidemiologic survey has revealed that it is the place where one spends early childhood that determines whether multiple sclerosis develops in later life. A study is now being planned to determine the latency of this disease and the most common age at which it is actually acquired.

One possible explanation for the long latency of this disease is that it results from a very slow developing virus infection. This thesis has received support recently through the Institute's studies which have demonstrated a transmissible agent in kuru, a condition that bears some resemblance to multiple sclerosis. Long-range studies involving animal inoculation with material from patients dying of multiple sclerosis are in progress.

An essential step in the solution of neuromuscular diseases is precise knowledge of the underlying biochemical defect, since most represent "inborn errors of metabolism." Two centers to study the fundamental physiological and chemical characteristics of muscle are being supported. Additionally, the Institute's attack could support a strong university-based clinical research center in addition to the intramural program for the study of the chemical and anatomical abnormalities of patients suffering from these diseases.

Knowledge of the fundamental mechanism in myasthenia gravis is progressing rapidly. The disease is clearly related to abnormalities of the thymus gland. Institute studies have now documented that thymectomy is a useful procedure, especially in young women. Studies of scientists in the intramural program of the National Institute of Allergy and Infectious Diseases are suggesting that an autoimmune process afflicting the thymus is a fundamental disorder,

and that abnormal amounts of thymic hormone may be responsible for permanent muscle damage in this disease. These findings could lead to an exciting new therapeutic approach to this disease. Meanwhile, therapy by thymectomy, by the use of ACTH, and by anti-cholinesterase drugs is increasingly effective.

The rising toll of death from accident and injury includes a large proportion who have experienced head and spinal cord injury. Laboratory studies within the Intramural Program are directed toward understanding the mechanism of action in factors causing brain swelling after head injury and the ways in which this destructive phenomenon can be modified. In addition, there is an active program for the study of the physical forces of injury--knowledge essential for the development of protective devices, as well as for the understanding of the nature of the injuries of the brain which occur.

A large program to study the physical characteristics of brain tissue and its response to injury has been established under contract. Also, four studies of excellence for the clinical investigation of head injury are in the planning stages throughout the country. Within the next 5 years at least one center will be developed for the study of spinal cord injuries.

Several Institute-sponsored conferences and workshops have been held and are planned to stimulate the expansion of brain tumor research. A Brain Tumor Task Force is preparing a testing protocol for a cooperative program involving several clinics. This program will evaluate therapy, particularly chemotherapy.

Biochemical or other objective methods for evaluating tumor growth are greatly needed. Recent studies on cholesterol metabolism of brain tumors suggest that this mechanism can be used as an index of tumor growth. This research is being continued and expanded.

The most impressive progress in brain tumor research has been in diagnosis. The development of new radioisotopes and equipment has made brain scanning a more economical and rapid means of diagnosis. Diagnosis by ultrasound has proved useful in clinical practice and is an extremely safe and rapid method. Investigators are busily engaged in refining ultrasonic techniques because improvement in the accuracy of this method is needed.

Cerebral edema accompanying the tumor can often cause more distress to the patient than the tumor mass itself. Researchers have found that corticosteroids can have a marked effect on this edema and will improve the patient's health although no change in the tumor takes place. However, how the steroids act and what causes the swelling is not known, but a relationship to the physiological processes of the "blood-brain barrier" exists. The NINDB supports several large research projects investigating the basic phenomena.

The Institute is supporting five multidisciplinary projects covering various aspects of human communication. These include an investigation of the fundamental processes of hearing at Princeton; auditory communication at the Central Institute for the Deaf in St. Louis; deafness and vertigo at the University of Chicago; causes of deafness at the Kresge Hearing Research Institute, Ann Arbor, Michigan; and voice production, psychology of speech

behavior, language acquisition, linguistics, and voice pathology at the University of Florida.

A specialized Information Center for Hearing, Speech, and Disorders of Human Communication has recently been established at the Johns Hopkins University. It is providing a review of the field and dissemination of information of significance to the scientific world.

A special subcommittee of the Council has completed an extensive analysis of progress and needs in the field of human communication. Its report is now being prepared and should provide useful guidelines for the development of a stronger national program in this field.

The Institute's rapidly growing attack on blindness is being led by the Council Subcommittee on Impaired Vision and Blindness. The exhaustive study completed by this group a year ago has provided a blueprint for this effort. Special task forces are concerned with refractive errors and with the study of pharmacological agents. These two areas are particularly in need of more rapid development.

The Institute's clinical eye research program is largely centered in 12 large multidisciplinary clinical research centers. To further extend these clinical research efforts, the Institute is developing a number of outpatient eye clinical research facilities.

The significance of advances of fundamental knowledge in vision research was highlighted this year when two of the Institute's grantees received Nobel Prizes for their contributions--Dr. Halden Keffer Hartline for studies of the physiology of vision, and Dr. George Wald for contributions to the understanding of the chemistry of the visual pigments.

Various offices serving the Office of the Director have aided substantially in advancing the mission of the Institute this past year.

The Office of Program Analysis has collected, indexed, and analyzed extensive materials which have facilitated Institute program plans and projections. Its scientific information file will serve all areas of the Institute and will include not only Institute research and training information of all types, but relevant efforts of other Government and non-Government agencies.

Under the direction of the Office of Program Analysis, the Neurological Information Network of the NINDB has matured and is now providing service to scientists throughout the world. The oldest of the centers, the Parkinson's Information Center at Columbia, now has a biweekly alerting service to meet the increasing demands for information. The Brain Information Service at UCLA has an established workshop program and has produced important reviews in appropriate journals. The Johns Hopkins University Information Center for Hearing, Speech, and Disorders of Human Communication is publishing a new periodical called Communication Disorders. And the Vision Information Center at Harvard is now well under way.

The Office of Program Analysis has also planned and directed a number of workshops and has prepared extensive reports in areas of vital interest to the Institute.

The Office of Public Information has expanded its comprehensive information and public relations program to serve a large public interested in the neurological and sensory area. An increased effort was made to reach more people engaged or interested in the health professions, including physicians, scientists, educators, members of Congress, voluntary health agencies, State and local health officials, and private citizens.

Institute publications now include 16 pamphlets in the "Hope through Research" series for the public; 12 brochures in the "Research Profile" series; 7 in the scientific monograph series; 4 in the special fact sheet series; and 3 others in special areas of public interest.

This past year, many articles were written by the Office of Public Information to fill a variety of press and journal needs and more than 100 reporters and feature writers were assisted in the preparation of materials relating to brain, eye, ear, and speech research.

A close working relation was maintained with more than 20 voluntary health agencies in the neurological and sensory field. Many of these were provided pamphlets, speeches, films, and exhibits, and the Office of Public Information assisted a number of the voluntary agencies with national and regional meetings.

Two new exhibits were developed and scientific and Institute program exhibits were shown at various national and international professional meetings.

This past year a new emphasis was given to radio and television. Sequences from several Institute films were shown on network programs, materials were made available for medical reports, and arrangements were completed for a series of radio spots relating to Institute programs.

The Office of Biometry, providing a central Institute source for service in statistics, mathematics, data processing, and computer technology, worked extensively with Institute scientists this past year on a wide range of studies. These included such intramural studies as one for the Medical Neurology Branch on the determination of muscle-tension contraction curves. In Collaborative and Field Research, complete staffing of statisticians was provided for the Perinatal Research Branch including computer-program and systems-design personnel. The analysis of data in the study of the relationship of early measles to later neurological symptoms for the Epidemiology Branch is another example of the service provided.

Servicing the Office of the Director, the Office of Biometry provided data for the review of the Perinatal Research Project by the Perinatal Statistical Ad Hoc Committee and also administered the Model Reporting Area for Blindness Statistics.

The Office of Biometry in addition aided the Institute's various programs by offering consultation to outside groups. These included the NANDB Council Subcommittee on Impaired Vision and Blindness which is developing an Eye Pathology Research Program; and the scientists developing a clinical trial to determine the effects of types of treatment for neurologically damaged children.

Assisting the Institute in its Inter-American activities is an office in Puerto Rico under the direction of Dr. Pearce Bailey. Highlighting these activities this past year was the Second Pan-American Congress of Neurology held in San Juan, October 1967.

Participating in the Congress were representatives from North and South America, the Caribbean, and from three European countries. Two joint sessions were held with the Seventh Latin American Congress of EEG and Clinical Neurophysiology and a Workshop on the Epidemiology of Epilepsy was held the day following the Congress.

One of many values of the Congress was the demonstration of the use of the bilingual scientific community in Puerto Rico for the coordination of activities and information between North America and Latin America. The Congress also helped to close the gap in Puerto Rico and Latin America between the relevancy of basic neurological science to clinical science in the teaching, research, and practice of neurology.

## ACTIVITIES OF THE OFFICE OF THE DIRECTOR

### The Office of Program Analysis

A primary objective of the Office of Program Analysis is to provide program information necessary for management decision on current programs and for future plans and projections. This includes complete information on all NINDB activities and their relation to the overall mission of the Institute and relevant research and training efforts of other Government and non-Government agencies.

### File of Scientific Data

To provide the necessary information, all of the research projects supported by the NINDB have been indexed and the terms punched on keydex cards. There are some 1,200 terms, including many drugs and substances, so that coordinated searches in considerable depth can be carried out.

The scientific information file has been strengthened in two important areas: an annual print-out is obtained from the Science Information Exchange of all of the research relevant to the mission of NINDB being done throughout the United States; and a listing of the terminated and newly initiated research projects of NINDB and non-NINDB support have been started. The continued analysis of these listings aid in more clearly identifying research trends within the various areas of neurology and the neurosciences.

This past year, a total of 26,442 items were handled for consideration for inclusion in the scientific information file; this continues to be an average of about 2,100 items a month. Some 7,200 items were either manuscripts or reprints which were the results of research. At the present time, these are unindexed but plans are under way in the next year to begin indexing these research results.

### Scientific Information Network

The Neurological Information Network of the NINDB has continued a very healthy and steady growth. The Brain Information Service at UCLA and the Parkinson's Information Center at Columbia University are both through their early growth processes and are providing service to an increasing number of scientists. It is of interest that the number of scientists provided service from the local scene at UCLA has leveled off, and the number of outside scientists serviced is outstripping the number of local users.

The Information Network not only has a large U.S.A. clientele, but has become highly visible in Europe and other countries outside of the United States where such services are far less common.

The Brain Information Service, through the workshop mechanism, has produced five important reviews which have been published in journals to reach the maximum audience. This is proving to be a very effective mechanism and has become extremely popular with the neuroscience community. These reviews are

produced not only by American scientists but by scientists from various parts of the world.

Equally impressive has been the progress at Columbia where the weekly alerting service has been changed to a biweekly service because of the enormous number of requests. Various kinds of bibliographic searches are continuing and an updating of the Parkinson's disease review has been completed. This particular review has been so popular that the Government Printing Office has had to put it through a special printing. It is being reviewed in several languages and requests to translate it have been received. However, because of the new edition, this has not been done.

The bibliographic service of the Parkinson's Information Center is indexing neurological literature for the National Library of Medicine and this has continued to be increasingly effective. It is serving as a model for other information centers for indexing for the Index Medicus.

One of the developmental problems of the Information Network was the possible physical linkages between the centers. This problem was studied on contract this past year by Auerbach, Inc., management consultants. Their report will propose a central office with computer linkage to the various nodes. Implementation of these recommendations will be started this year.

The Information Network is becoming a model experiment which is being observed and examined closely by the other information and communication efforts being conducted throughout the country.

This past year, the Program Analysis staff was asked to present a special panel of members of all of the information network centers at the American Documentation Institute. Also, various members were key speakers at several other important meetings throughout the country.

A combined network exhibit is being planned for the major professional societies for next year.

Reports and Workshops

The development of Epilepsy Abstracts was worked out by the Office of Program Analysis through contracts with Excerpta Medica. Because this is intimately associated with the epilepsy program, the Head of the Epilepsy Section was designated as Project Officer, but the Office of Program Analysis provides the Assistant Project Officer and has been very closely associated with this project which is being well received throughout the country.

The Cerebral Vascular Disease alerting service provided by the Mayo Clinic under contract continues to be useful and desirable. An evaluation conducted by the Joint Council Subcommittee for Stroke has substantiated its value.

Many special reports have been generated throughout the year in response to various Institute needs. Those of special interest were: the NINDB vision programs as back-up material for hearings on an Eye Institute; the statistics

of multiple sclerosis, done at the Mayo Clinic; and a study of spinal cord research. Using the system devised by the Office of Program Analysis, the Department of Health, Education, and Welfare has put together its sponsored mental retardation research in a way which promises much more effective reporting.

The file of information about NINDB-supported trainees is being edited and put onto punch cards to allow machine analysis and comparison with other data basis. This analysis will be completed in the coming year.

Workshops were planned and held by the Office of Program Analysis on Brain Tumors, Inborn Errors of Metabolism, Cerebrospinal Fluid Physiology, and Trauma as a Cause of Mental Retardation. A number of these have resulted in extensive reports which will be widely disseminated.

## The Office of Public Information

The major mission of the Office of Public Information, NINDB, is to inform the public of the latest neurological and sensory research advances. Since millions of Americans are victims of such long-term neurological and sensory disorders as cerebral palsy, multiple sclerosis, epilepsy, stroke, parkinsonism, head injury, deafness, and blindness, there is a deep interest in all new research developments which give hope for a healthier future.

In 1968, the Office of Public Information expanded its comprehensive information and public relations program to meet these demands. More information was disseminated to the professional and lay communities, particularly through Institute publications. An increased effort was made to reach more people engaged or interested in the health professions. These people make up a large audience which includes physicians and scientists, educators, members of Congress, voluntary health agencies and foundations, State and local health officials, and private citizens.

### Publications

More than 120,000 copies of various types of brochures were distributed through the publications program. The Office of Public Information reviewed and improved its distribution methods and has attained considerable success in reaching a larger audience through increased cooperation with voluntary agencies. Through these efforts more people in both the professional and lay audiences are now becoming acquainted with the NINDB research effort.

Among the new and revised publications of 1968 were nine published in the "Research Profile" series (Director's reports to Congress). In addition, ten reports were written for later publication in the series. In the "Hope through Research" series, three new pamphlets were published. These were: "Dizziness," "Learning Disabilities due to Minimal Brain Dysfunction," and "Spinal Cord Injury." They became the 14th, 15th, and 16th in this popular lay series. Three other lay pamphlets were published in revised versions.

Four monographs in the Institute's scientific monograph series were prepared for publication and distributed by the NINDB Information staff. They were "Vision and Its Disorders," Monograph No. 4; "Refractive Anomalies of the Eye," No. 5; "Drug Trials for Headache: Principles and Methods," No. 6; and "The Public Health Aspects of Hearing, Language, and Speech Disorders," No. 7.

Major assistance in the development of a new periodical, Epilepsy Abstracts, was provided by the Office of Public Information. Now being issued each month by this Office, the publication offers abstracts of approximately 200 articles on epilepsy each month, and covers most of the relevant world medical literature on epilepsy.

An illustrated brochure on the Institute's Laboratory of Perinatal Physiology in Puerto Rico was published. Copies in both English and Spanish were

distributed at the Second Pan American Congress of Neurology, held in San Juan, October 22-28, 1967. A second printing is being distributed by the United Cerebral Palsy Associations.

At the request of the National Association for Retarded Children, the Office of Public Information published a brochure on measles encephalitis. It was designed to inform parents about the possible serious consequences of measles and to encourage them to have their children vaccinated.

More than 10,000 copies of a reprinted leaflet on amyotrophic lateral sclerosis was distributed by the Institute and two voluntary health agencies: the National Multiple Sclerosis Society and the Muscular Dystrophy Associations of America.

Six issues of the "NINDB Review" were written and published for distribution to voluntary health agencies and NANDB Council members. Also, the Office staff compiled and published a list of voluntary health agencies working to combat neurological and sensory disorders. In addition, the Office prepared and published two publication checklists--one for the general public and a second for professional use. The checklist for the general public lists 23 publications; 27 publications are available for professional use.

Public Inquiries

In fiscal year 1968, the Office of Public Information received requests for information on all the leading and many of the more obscure 200 neurological and sensory disorders. As in previous years, the largest number of inquiries related to eye disorders. This year, there was great interest in the disease, amyotrophic lateral sclerosis. Interest was specially great on the Hill where Congressmen became most sympathetic to the plight of a former member with the disease.

At least 6,000 written public inquiries were received by the Office of Public Information. More than 1,200 of these required individually prepared letters in answer to specific questions. These included over 300 Congressional inquiries. The remainder were answered with printed materials. In addition to questions answered by letter and published materials, more than 100 each month were answered by phone.

Press and Periodicals

More than 100 reporters, feature writers, editors, and broadcasters requested Institute information. The Office prepared and distributed 14 press releases and announcements. Twelve articles were written for the NIH Research for Health series and sent to daily and weekly newspapers. These included such subjects as cataract, glaucoma, mongolism, shingles, epilepsy, amyotrophic lateral sclerosis, hearing loss, little strokes, retinitis pigmentosa, diabetic retinopathy, and disorders of the cornea. A total of 34 stories were written for the NIH Record.

Interviews were arranged with the NINDB Director or other scientists for articles and stories used in scientific and lay press. Included were:

U.S. Medicine, Medical World News, Today's Health, Drug Research Reports, JAMA, Medical Tribune, Changing Times, Roche Image, National Observer, Parade Magazine, Good Housekeeping, McCall's, Parents' Magazine, Reader's Digest, Time, Washington Post, and many others.

In addition to materials and assistance supplied to the journals and press, articles were written specifically for PHS World and Volta Review.

Press Conferences and Meetings

Wide publicity was given the Institute as a result of two press conferences arranged by the Office of Public Information. The first one was held on September 14, 1967, following an Institute-sponsored conference on measles virus and subacute sclerosing panencephalitis. Four of the scientists from the SSPE meeting participated in a press briefing covered by a group of science writers.

On April 29, 1968, a second press briefing was conducted by the Office of Public Information on the Collaborative Perinatal Research Project. The NINDB Director and two Project scientists briefed medical writers at the Department on recent findings from the 10-15-year ongoing mother-child study. As a result of the briefing, stories appeared in a large number of newspapers and medical journals around the country.

In addition to these press meetings, the staff of the NINDB Office of Public Information assisted in the Second Pan American Congress of Neurology, held in San Juan, Puerto Rico, October 22-28. Two exhibits were designed for the meeting, one in Spanish; a brochure on the Institute's program in Puerto Rico was prepared in both English and Spanish; and a publications program was organized as well as a 3-day movie program for the Congress.

TV and Radio

Sequences from the NINDB film, "Rhesus Monkeys of Santiago Island, Puerto Rico," were provided three TV networks. NBC used excerpts for its Animal Secrets series; CBS showed parts of the film on its 21st Century program. Time-Life Nature Series used a 5-minute sequence in a film on animal behavior. Printed materials and photographs on head injury and eye disease were provided for Dr. Watts' program, The Doctor Reports.

Arrangements were begun for 20 radio spots on the Institute program. Two TV institutional spot announcements are being developed.

Reports and Special Documents

Office staff wrote or assisted in the preparation of various Congressional materials required by the Director during appropriations hearings. These included the opening statement, 10 special reports, a document "Highlights of Research" summarizing 11 areas, and other materials.

A large number of other special reports were written during the year. One of these described the program of the Office of Public Information for the past 10 years and plans for the future.

During the year, 48 journals were scanned on a weekly or monthly basis, and 43 research weeklies, as well as program development items, were prepared for the Director, NIH, and the Assistant Secretary for Health and Scientific Affairs using grantee or Institute scientists' findings.

Arrangements were completed for many Institute lectures, including the preparation and distribution of flyers.

Speeches and Special Statements

Nine speeches were written for delivery by the NINDB Director, Assistant Director, and the Surgeon General. Suggested messages or proclamations from President Johnson, requested by leading voluntary organizations marking special events, were written on numerous occasions.

Films

A shooting script was approved and arrangements made for a contract for production of a film on current research on the early identification of neuro-motor-sensory deficits in young children. A film on NINDB's research program is in the planning stage.

Exhibits

The Office arranged and manned exhibits at such national meetings as American Academy of Neurology, American Neurological Association, American Academy of Ophthalmology and Otolaryngology, American Speech and Hearing Association, and the Second Pan American Congress of Neurology.

This past year, two new NINDB exhibits were developed: one presented the Institute's program in the Western Hemisphere to the Second Pan American Congress of Neurology; the other depicted the Institute's Stroke Program to the Regional Medical Program directors. A scientific exhibit on multiple sclerosis research is being produced cooperatively with the National Multiple Sclerosis Society. An exhibit presenting the Institute's Information Network is being developed.

Cooperation with Voluntary Agencies and Foundations

The NINDB Office of Public Information worked closely with the National Committee for Research in Neurological Disorders and its many members--some 20 national voluntary health agencies and 10 professional societies in the neurological and sensory area. Examples of such cooperation included the production of cooperative scientific exhibits, assistance with the distribution of films, cooperation in the production and distribution of pamphlets, and assistance with national and regional meetings of various voluntary agencies. These included special projects this year with The Deafness Research Foundation, Epilepsy Foundation of America, Muscular Dystrophy Associations of America, Inc., National Association for Retarded Children, National Multiple Sclerosis Society, National Paraplegia Foundation, National Society for Crippled Children and Adults, Paralyzed Veterans of America, Inc., and United Cerebral Palsy Associations, Inc.

## Inter-American Activities

### Goals

The goals of the Inter-American activities in Puerto Rico are: 1) investigate the research and training potential in Puerto Rico and Latin America as they relate to NINDB; 2) contribute to the growing NINDB programs in neurological science in Puerto Rico; 3) advance, integrate and coordinate NINDB activities in Puerto Rico as they relate to NINDB activities in the U. S. mainland and other sectors of the Americas; 4) in a staff capacity, help develop creative and meaningful liaisons of NINDB programs in Puerto Rico with their counterparts in the Americas, and 5) thus aid in the development in Puerto Rico of a creative setting and ambiance for a true Inter-American interchange of neurosensory information, ideas and developments. In this way, the NINDB investment in Puerto Rican neurological science can be reinforced by having the Puerto Rican establishment in neurological science function as an important connecting link of scientific exchange between the neurological communities of the Americas.

For NINDB programs in Puerto Rico to constructively fulfill their mission, Puerto Rican neurological science should have a creative identity as is envisaged in its growth as a central focal point for an interchange of knowledge in the neurological sciences between North and Latin America.

To aid in the achievement of these goals as they work to the benefit of NINDB's involvement, is the central purpose of the Inter-American activities. These aims have not and do not in any way relate to the solicitation or procurement of NINDB grant awards.

### Methods

Progress toward the attaining of these goals has been made by: 1) evaluation of NINDB neurosensory programs in Puerto Rico through interviews with Puerto Rican officials and department heads, consultations, seminars and lectures at the Puerto Rico School of Medicine and the Medical Center; 2) visits to all academic and research medical facilities in Puerto Rico and in Latin America when consonant with NINDB policy; 3) extensive correspondence with leading neurologists and neurological scientists from Latin America; and 4) offtime participation in the activities in the Puerto Rico academic and voluntary agencies programs that are dedicated to neurosensory disorders

The Chief of Inter-American activities has a background knowledge and experience for participation in such activities. He is, for example, Professor of Clinical Neurology at the School of Medicine, University of Puerto Rico; an active member of the Puerto Rico Medical Association; member of the Advisory Board of the Section of Psychiatry, Neurology and Neurosurgery of the Puerto Rico Medical Association. He is the

founder of the Puerto Rico Academy of Neurology and Alternate Delegate from Puerto Rico to the world congresses of neurology; Honorary President of the Second Pan American Congress of Neurology; honorary member of all Latin American neurological societies and recipient of the highest government order of the country of Peru (Comendador de la Orden "El Sol del Perú".) He has command of both Spanish and French. He is on the Medical and Research Advisory Boards of the local voluntary societies of epilepsy and muscular dystrophy.

Activities in Fiscal Year 1968

The fiscal year 1968 Inter-American activities have increased professional competency and imagination in NINDB-supported neurosensory programs in Puerto Rico despite losses to the doctor draft; closer clinical and basic science coordination despite financial curtailments; and the establishment of closer links between North and Latin American scientists in neurosensory research despite travel restrictions and spiraling inflation in many Latin American countries.

Among the more highlighted developments of Inter-American activities in fiscal year 1968 was the bringing of the Second Pan American Congress of Neurology to San Juan at the end of October, 1967.* The inducements used to have the Second Congress in San Juan were its strategic location - an approximate midpoint between North and South America; its progressive scientific outlook; its pleasant setting and modern facilities, but above all, the bilinguality of its scientists - a fact which permitted the Congress participants to share their experiences, hold frank discussions without embarrassment, and indulge a camaraderie seldom seen before.

The Congress program gave exposure to such topics as neurology and space research, the latest concepts in neurological education in the U. S. and a sophisticated scientific program dealing with such topics as the nature of nerve conduction in its various aspects on cellular and subcellular levels, the epidemiology of neurological diseases and the neurology of behavior. The participating authorities on these subjects came from the United States, Canada, Mexico and South America. It was a neurology showcase for all Latin American neurologists to see and, indeed, it was more than that, for they participated and added to the richness of the Congress.

The congressionists came from North America and from the countries of South America and the Caribbean, as well as from three European countries (England, France and West Germany) who happened to be in the neighborhood. Two joint sessions were held with the Seventh Latin American Congress of EEG and Clinical Neurophysiology which occupied the same headquarters and facilities as those of the neurological congress and thus added to the scientific cosmopolitanism of the sessions.

* The First Pan American Congress of Neurology was held in Lima, Peru, in October, 1963.

The central core of the Congress program was augmented by sessions devoted to free communications, as well as a program of scientific exhibits, including two from the NINDB Perinatal Laboratory, as well as an NINDB program exhibit reinforced with numerous informative brochures and monographs provided by the Chief of the NINDB Information Office. A continuing program of motion pictures, mostly from NINDB, but some from voluntary health agencies, was presented. Some were narrated in Spanish for the Latin American participants. A printed volume of Congress proceedings is now being prepared under the editorship of the Chief of Inter-American activities. It will be printed in Puerto Rico and supported from local sources.

A sense of satisfaction came from the fact that the greater portion of the cost of the Congress was not borne by Federal grants, but by Puerto Rico itself and Congress registrations, the Department of State (Puerto Rico), voluntary contributions, and the local epilepsy society. There is, it appears, a growing awareness in Puerto Rico, aided and abetted to some degree by this Congress, that the burden of scientific events of this kind should be borne for the most part by the island itself - a sort of "operation bootstrap" applied to science parallel to its application in economics.

The Congress, as a whole, can be termed a success despite shortages in money and personnel, and despite travel restrictions because of balance of payment problems and inflationary trends. Its principal symbolic value resided in image-making and the closing of important communication gaps. Before the Congress, many Latin American scientists had only a hazy image of NINDB and almost no image at all of Puerto Rico and its potential in neurological science, which was practically unknown to them. Furthermore, the Congress helped diminish the separatism prevailing between North and Latin American neurology and it also helped to close the gap in Puerto Rico and Latin America between the relevancy of basic neurological science to clinical science in the teaching, research and practice of neurology. Finally, the Congress demonstrated the value of the bilingual scientific community in Puerto Rico for the coordination of activities and information between North and Latin America.

The day following the Second Pan American Congress of Neurology, Inter-American activities conducted a Workshop on the Epidemiology of Epilepsy which it had planned and organized in coordination with the Office of the NINDB Director. This was a practical situation, inasmuch as it afforded an opportunity for parties interested in this subject who had attended the Congress to stay over and convene with others in order to share knowledge and evaluate methodologies in this important problem whose systematic study had been too long overdue. The NINDB Director opened the sessions while the President of the Second Pan American Congress served as moderator. The other members of the 20-member panel consisted of epidemiologists, neurologists interested in epilepsy, and a geneticist. The Latin American participants came from Brazil, Chile and Mexico. The Workshop proved fruitful from the standpoint of initiating steps for the attack on a neglected program which it is hoped would have a follow up. It opened the way for the conferees to return to the institutions of their countries, speak about the Workshop and give further consideration to the problem.

The remainder of the Inter-American activities during fiscal year 1968 comprised a further development of projects undertaken in previous years using the methods described previously. A major obstacle to be overcome in the further development of neurological science in Puerto Rico and its Inter-American coordination, is the relative isolation of the sciences basic to clinical neurology. Part of this is conceptual, part due to the need for greater graduate school facilities, part due to the geographic separation of the pre-clinical studies where most of the research is done in the Medical School in Old San Juan, from the clinical activities which are all conducted at the Medical Center in Rio Piedras. There are both bright spots and gaps in the basic science activities, but even the bright spots are not well integrated with the program in clinical neurology. The amelioration of this situation which is progressing slowly must project its final fruition to future years.

As a result, plans and proposals for making Puerto Rico neurology an instrument for training Latin American neurologists in the basic neurological sciences have not yet sufficiently matured. More progress has been made in ophthalmology where during this fiscal year a course is operating for the training of Latin American ophthalmologists in sciences basic to ophthalmology.

## Office of Biometry

The Office of Biometry, NINDB, was established officially on August 28, 1967. Certain personnel, however, were detailed to this Branch in April, 1967, so that the following reports the activities of the organization for the entire period July 1, 1967 - June 30, 1968.

The Office of Biometry consists of the Office of the Chief and of four sections, each with a specific mission. The primary objectives of the Branch are to provide all arms of NINDB with a central source on which to draw for complete service in statistics, mathematics, data processing, and computer technology.

The following is a brief description of projects in which the Office of Biometry provided consultation and computational service in the design, analysis, and evaluation of researches and experiments by the clinical, basic, and collaborative-research scientists of NINDB. The listings are by the arm of NINDB to which service was given.

## Intramural Research

### Surgical Neurology Branch

1. Analysis of response data from electrode stimulation in epilepsy cases.

2. The application of curvilinear regression techniques to the analysis of data arising from a head-injury research project relating to cerebro spinal fluid.

3. Development of a mathematical model for study of induced head-injury, including analysis of angular acceleration and angular velocities of concussed and non-concussed monkeys with whiplash impact, collar plus impact distributed load.

4. Development of data-collection vehicles and flow system for chimp observation study.

5. Review of all analyses done in 1964 on data developed from observation of 150 epilepsy patients.

6. Instruction on use of Central Data Processing Branch's program for multivariate analysis of variance.

### Medical Neurology Branch

1. The mathematical problem of determining muscle tension-contraction curves. The relationship between muscle tension and contraction is shown to be represented by exponential curves.

2. The analysis of data from counts of muscle fibers, from muscles with carrier dystrophy, definite or probable vastus, etc. A variety of parameters were used to summarize and characterize the data.

3. The analysis of data obtained from study of fiber diameters and fiber retractors was made by means of correlation and regression analyses, and by other statistical methods.

4. The analysis of data on muscle stress-strain, leading to the fitting of exponential-type curves.

5. Analysis of data from muscle tension experiments, with tests for symmetry, and variance analyses.

6. Analysis of data from experimental use of various chemicals on muscles, and measurement of differences in response using correlation, regression and covariance analyses.

7. Analysis of responses of muscles, in resting and non-resting states, to various drug applications.

Laboratory of Perinatal Physiology

1. Design of systems for maintaining an inventory of monkey colonies and in preparation for analysis of data deriving from experiments on primates.

2. Development of data-collection vehicles and systems for retrieving data f pathologist (Johns Hopkins) in consultation with laboratory.

Laboratory of Neurophysiology

1. Consultation on design of a research relating to physiological study of vision.

Ophthalmology Branch

1. Analysis of tonometry data, with and without drugs and before and after duugs.

2. Interpretation of results in continuing project to study relationships between aqueous inflow, intraocular pressure and venous blood pressure in the eye.

3. Design and analysis of a study of correlations between eye-pressure differences (differences between blood pressure and intraocular pressure) and aqueous inflow.

4. Consultation and computation (partial and multiple correlation, exponential and curviliniar regression, regression analyses) for study of ocular pressure and the change on the volume of fluid of the anterior chamber of the eye.

Laboratory of Molecular Biology

1. Consultation and statistical assistance in classifying 45 bacteria mutant strains based on their growth in three cultural media, and later in five different culture media. A multivariate analysis of variance procedure was employed and a computer program was prepared for computing the MANOVA. This has led to a continuing research toward the development of an optimal statistical procedure for classifying bacteria mutants on the basis of their growth responses in the presence or absence of certain parameters in the metabolic cycles.

Electroencephalography Branch

1. Development of sampling procedures and interpretation of test results in a study for assessing the incidence of electroencephalographic eliptiform discharges and development of seizures.

Collaborative and Field Research

1. Evaluation of a statistical technique used in the study of the relationship between serum-cholesterol levels and certain eye diseases.

2. Provided complete staffing of statisticians, computer programmers, and systems-design personnel to the Collaborative Project on Epilepsy. Provided consultation to principal investigators, measured quality of data, handled all data retrieval and computer functions, and analyzed statistically data arising from program.

3. Developed techniques and prepared programs for computation of maximum likelihood estimates of gene frequencies in blood grouping.

4. Reviewed and proposed revision of the proposed study of the anti-convulsant properties of Albutoin, to be undertaken by the New Castle State Hospital, New Castle, Indiana, in regard to the experimental design, the measurement of the response variable, investigation of side effects, etc.

5. Preparation of code for head-injury study, so as to provide simplified processing and retrieval of data.

Perinatal Research Branch

1. Provide complete staffing of statisticians, computer programmers, and systems-design personnel to the Collaborative Project. Provide consultation to principal investigators and scientists at 14 university hospitals collaborating in Project, handle all data retrieval and computer functions, and analyze statistically data arising from program. Staff quality-control unit to maintain level of data collected in study.

Epidemiology Branch

1. Design of a sampling plan for the epidemiological investigation into the etiology of uveitis.

2. Analysis of data relating pulmonary disease to neurological symptoms.

3. Design of a study to measure the effects of surgery on patients suffering unilateral Parkinsonism.

4. Analysis of data in the study of relationship of early measles to later neurological symptoms.

5. Development of structured data-collection vehicles for the epidemiological study of amyotropic lateral sclerosis on Guam.

6. Design of investigation into Parkinsonism Dementia.

7. Analysis of data measuring the extent of hearing loss in children.

8. Design of study of Dystonia Musculorum Deformans.

9. Design of study of convulsions, and development of data-collection vehicles.

10. Analysis of data comparing various titre levels in patients with multiple sclerosis with those in several groups of close controls.

11. Design of study investigating relationship of tumors to ophthalmological disorders.

12. Tabulation of census of births on Guam during 1965, with related data.

Extramural Programs

1. Completed preliminary review of needs for data processing and retrieval in NINDB grant area. Outlined the final concept of data files and variable files for Extramural Program.

Office of Director, NINDB

1. Maintain the Model Reporting Area (MRA) for Blindness Statistics. Coordinate the activities of the 15 states currently involved in the program, and handle centrally the complete statistical and data processing functions of the program.

2. Provided complete service in production of data for review by Perinatal Statistical ad hoc Committee, and had representatives at all meetings of Committee to interpret data, meet Committee's requests for material for evaluation, etc. Part of the service to this Committee required the development of more than a hundred thousand cross-tabulations, and a large number of separate studies and analyses were made for the Committee.

Organizations and Individuals Outside NINDB

1. Statistical advisers to committee developing large-scale publications of data collected in course of Collaborative Project on Cerebral Palsy, Mental Retardation, and Other Neurological and Sensory Disorders of Infancy and Childhood. Completed layouts of Volumes I and II, and have completed programs for data processing to be undertaken following review of layouts.

2. Statistical advisers to committee developing Eye Pathology Research Program, and proposed central source for data arising from program.

3. Statistical advisers to university scientists engaged in clinical and laboratory studies of various aspects of uveitis.

4. Statistical advisers in proposed clinical trial to determine effects of Doman-Delacato treatment of neurologically damaged children.

5. Designed study to develop method for screening pregnant women at time of first visit to obstetrician for difficulties in labor and/or risk to fetus.

6. Designed study to determine whether head circumference at age of one year is valid predictor of subsequent mental retardation.

7. Statistical advisers to Perinatal Study undertaken in Warsaw, Poland. The Office of Biometry will process the data developed from this study, and will provide the statistical support in the data analysis.

8. Designed study to determine antecidents of mental retardation, during early course of infancy.

9. Designed and analyzed study of effects of maternal weight gain during pregnancy on birthweights of infants.

Methodological Research

1. Development of test of homogeneity for k binomial samples when the expected number of "successes" is very small. The problem arises in the testing of inter-examiner differences of standard tests in which the relative frequency of examinations varies substantially by examiner, with some examiners making few tests, and where the expected number of abnormal results is small.

2. Development of methods for dealing with missing values in multiple regression analysis. Paper to appear in Biometrics in September 1968.

3. Establishment of objective rules for selecting transformations of responsive variable in quantal assays.

4. Development of method of comparing two groups, where each group consists of matched pairs, with continuous outcome data.

5. Development of a check-out program for multiple regressions and discriminant functions via computer.

6. Estimation of stimulation-response relationship from individual quantal assays.

7. Development of optimal statistical procedure for classification of bacteria mutants.

8. Development of programs of computation of maximum likelihood of gene frequencies in blood groupings.

9. Development of optimum procedure for separating normal and abnormal babies in a mixed population based on gamma-globulin levels in blood.

10. Development of the curvilinear distribution of the theoretical relationship between intraocular pressure and the change in volume in the anterior chamber eye fluid.

11. Development of the facility to use a remote teletype system to produce mathematical computations and other tabulations on a computerized basis. Eight people have been trained in the use of programming techniques associated with this system, and four clerks have been trained in the use of the machine so as to provide the facility needed.

12. Development of a series of utility programs for the IBM 360 to permit simple and quick turnaround on special computations and listings.

13. Development of systems to meet data processing needs of all arms of NINDB. These have led to the contracting for and procurement of Express III, a software system for rapid processing of data for the CDC 3600 and the IBM 360/50, and contract proposals for a CalComp CRT Plotter and Software to produce large volumes of charts for analytic use.

Future Plans

The Office of Biometry plans to continue to provide service to all NINDB programs and units, and to outside scientists engaged in research in areas of the Institute's interest, in much the same fashion that it has been doing. It is expected that the demand for statistical and data-processing services will grow as the fact of the existence of the Office of Biometry becomes more widely known; the relatively recent establishment of the Office of Biometry has left some arms of NINDB unaware what services are available on request.

Problems

The major problem of the Office of Biometry is staffing; it is extremely difficult to attract qualified personnel, in view of the heavy demand for biometricians by major industry. The Office of Biometry managed to recruit three Ph.D.'s and several other well-qualified people in the last year; the expected expansion of its workload will require a continued success in recruitment.

Man Years of Work

Total: 40.5

Professional: 25:5

Others: 15

Publications

Members of the Office of Biometry were authors or co-authors of the following:

1. Organic heart disease, Amer. J. Obstet. Gynec. 98:871-876, July 1967.

2. The placentae weight-birthweight relationship. Develop. Med. Child Neurol. 9:160-171, April 1967.

3. Birthweight-outcome relationships in infants term by gestation. APHA October, 1967.

4. Marginal Sinus Rupture, Amer. J. Obstet. Gynec. 99:862-866, March 1968.

5. Missing values in linear multiple discriminant analysis, Biometrics, Sept. 1968.

6. Proceedings sixth annual conference of Model Reporting Area for Blindness Statistics. U.S. Dept. of Health, Education and Welfare, Public Health Service Pub., Wash., D. C., 1967.

EYE RESEARCH FOUNDATION (PH 43-66-91)

Title: A Genetic and Histological Study of Deaf White Cats

Contractor's Project Director: Drs. Carl Richard Cavonius and Eugene P. Rubacky

Project Officer: Dr. Eldon L. Eagles, NINDB and Dr. Kenneth S. Brown, NIDR

Objectives: To study the mode of genetic transmission of deafness and pigment changes in the cat and in particular to evaluate the existence of one or many genes causing the defect; to record the anatomic, developmental, and functional changes which occur in the eye and ear; to relate the anatomic and functional changes to more basic gene actions.

Methods Employed: Through selection of animals with white fur and one or two eyes lacking iris pigment from domestic farm cat stock, a colony of about 90 animals has been established. The hearing function is being evaluated by four methods:

1. In all animals, by the detection neurophysiological signals (probably cochlear microphonic) from the scalp of anesthetized animals using a phase sensitive detector.
2. In all animals, by use of a standard intensity tone and evaluating behavior in awake animals.
3. In selected animals, through operant conditioning using positive reinforcement.
4. In selected animals, detection of cochlear microphonic and eighth nerve potentials from an electrode placed in the round window through a surgical approach just prior to the preparation of the ears for histological study.

Ears of animals with detected deviations from normal response patterns are prepared using wet celloidin technique modified from Guild and Fernandez (see attached reprint). Every tenth section is stained with hematoxylin and eosin for analysis by the method of Guild and Schuchnecht (Acta Otolaryng. 58: 377-397, 1953). Woelke and P.A.S. stains are done on midmodiolar sections of each ear sectioned.

The eyes of the animals are classified individually for pattern of iris pigmentation and presence or absence of tapetum. A series of eye sections showing the variation and development of iris and tapetum in the presence of the genes under study have been obtained. Electroretinograms of eyes with and without tapetum have been collected. These studies are done to attempt to differentiate possible variants of this condition.

The coat color pattern of animals is examined and diagramed at one or two weeks of age and at time of neurophysiological study to evaluate the variation in the pattern and changes that occur with maturation.

Applying the test hypothesis that this trait is a homogeneous one due to a single dominant gene, a mating scheme is being employed to progeny test selected sires and dames from the original phenotypically selected stock. Pedigreed Persian stock has proven to be unproductive and is being abandoned as a research line.

Major Findings:

1. The hearing loss of these animals is highly variable and is associated with several qualitatively different behavioral neurophysiological and anatomic patterns.
2. The hearing loss is progressive in the newborn period in some kittens.
3. The behavioral and nonbehavioral tests show good correlation and suggest that the presence of detectable voltage on neurophysiological testing is an excellent qualitative and quantitative determinant of sound controlled behavior in the cat.
4. Three patterns of histological abnormality have been observed in the white cats. Collapse of the membraneous portion of the inner ear with primary involvement of the ductus cochlearis and saculus and always the ductus reuniens is the most common pattern. This is usually a total collapse and Reissner's membrane obliterates any evidence of a differentiated organ of Corti if it ever existed. The vestibular and utricular parts of the membraneous labyrinth are usually not involved and there is not evidence of any infection or disease of the middle ear. This pattern may be either unilateral or bilateral and is associated with lack of voltage at any frequency tested in the electrophysiological studies

   The second pattern is one of partial collapse of all of the endolymph containing structures. The degree is highly variable and may be unilateral but is associated with the appearance of degeneration of a previously developed organ of Corti. It may be patchy within one cochlear so that part of the organ of Corti appears normal. The characteristics of this pattern is a balling up of the tectorial membrane with varying degree of cellular encapsulation. This pattern is often associated with histological evidence of middle ear infection and on occasion history of severe respiratory disease prior to death. This second pattern occurs more often in older animals purchased from the outside than from colony born animals. When bilateral, it is associated with a pattern of behavior in which the animal can be sound controlled but only at very high intensities. The neurophysiological pattern shows a severe decrease in amplitude of response but a normal slope for the voltage increase with increase in sound intensity in the tested ranges.

   The third pattern of cochlear defect observed is one of simple architectural malformation in the organ of Corti. This has been observed in one animal involving one ear for about one-third of the

length of the organ of Corti. Two offspring of this animal have been examined and the defect was not transmitted although there was some quantitative difference in the cochlear size of one of the offspring. This defect was not associated with any dilation of the endolymph containing passages.

5. A comparison of the frequency of phenotypic patterns of offspring produced in the colony and those patterns observed in the wild suggest that the white phenotype is under severe handicap particularly where it is expressed bilaterally with respect to eye structure. If this severe selection is present then the frequency observed must be maintained by a combination of several mechanisms, e.g. favorable selection by man for white, several different genes producing the same defect with low mutation rates for each, and/or a gene with a high mutation rate.
6. The electroretinograms of the blue eye lacking tapetum are of normal pattern but are lower in amplitude than pigmented, tapetal eyes. The irises of nontapetal blue eyes are kept in a wider open position than pigmented tapetal eyes. These findings suggest that the nontapetal eye would be less functional in low intensity situations.
7. Animals with two blue eyes are more likely to be deaf than animals with one blue eye or two pigmented eyes although there is no directional association of the blue eye and deaf ear in unilateral deaf and heterochromic animals.
8. Examination of the fundus shows that the tapetal reflection develops normally about the third week so that funduscopic examination can be used to identify potentially abnormal eyes before iris pigment is definitive.
9. Histological sections of the eyes suggest that the tapetal cells are present in the defective eyes but that they do not contain the highly reflective material normally found.
10. Examination of the pigment pattern distribution in the hair of the cats suggests that the pattern is controlled through mechanisms similar to those suggested by B. Mintz (PNAS 58: 344-351, 1967). Several patterns of pigment have been observed among the offspring of animals originally selected as being phenotypically similar. It has been possible to remove some of these from further study as extreme variants of the spotted phenotype rather than the gene resulting in deafness, white hair and blue eyes.
11. The operant conditioning using positive reinforcement gives thresholds of hearing for noraml animals at least as low as that reported using aversive conditioning and possible better in the range up to 64 KHz. The white cats show several abnormal behavioral patterns. Some cannot be trained to sound controlled behavior at any sound intensity up to the limit of the equipment (100 db) although responding well to other types of stimuli. Some respond with sound controlled behavior only at very high sound levels but have good accuracy and consistency above threshold. Some show irregular response patterns with evidence of learning and lowering threshold. This latter group appears to contain some animals with unilateral losses who have been observed to learn to position, probably to maximize the sound input. Some other animals in this group are generally erratic in behavior and have been eliminated from further testing.

Significance to NINDB Program and to Biomedical Research: This study of the multiple changes produced by the action of a single major gene has demonstrated a high degree of variability in structure and function in what had previously been thought to be a nomogeneous entity. This has implications for the human analog of this condition Waardenburg's syndrome. Relatively minor and unilateral losses of function have been observed in the cats suggesting that similar defects might occur in humans. The variability of these animals also provide a working base for a variety of experiments on specific aspects of the hearing mechanism. The variations in tapetal structure provide a potential base for the study of tapetal function, embryology, and biochemistry.

Proposed Course of Project: The quality of the electrophysiological hearing testing procedure has proven to be such that several quantitative discriminations are possible. Since performance of this type of testing takes excessive staff time, some further automation of testing is needed. The range of frequencies now available for the neurophysiological testing is limited because of electrical interference with the receiver generated by the high frequency source. Further work is needed to extend the electrophysiological test frequency range to match the behavior testing. Studies after unilateral ablation will be used to evaluate one ear in isolation by both neurophysiological and behavioral techniques. Behavior testing has proven to be a very powerful tool but is expensive of both time and effort. The use of this method will be limited to selected animals with intermediate degree of hearing loss as detected by less expensive testing methods.

Genetic study of the animals will be directed at the short hair domestic animals. The major program of the breeding plan is backcrossing affected to unaffected animals to test for homozygosity of the genes in question. The animals who are of known genetic constitution and no longer of breeding value will be used in collaborative studies where possible. Further examination of the histological specimens from animals that have been fully tested will add to the degree of clinical pathological correlation on the congenital and infection associated defects. The presence of the confounding effect of infection makes increased stress on isolation and disease control in the colony as well as placing increased value on the medical records being kept of each cat as a part of the developmental record. Further study of the distribution of the phenotype in the wild will be coupled with larger samples of colony experience. Development of hearing and its histological correlates will be studied in the newborn period as well as the histology of tapetal development.

Serial No. NDB (OD) - 61 B/BS 833
1. Office of Biometry
2. Section on Blindness Statistics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Model Reporting Area (MRA) for Blindness Statistics

Previous Serial Number: NDB (CF) - 61 B 833

Principal Investigator: Paul H. Anderson

Other Investigators: James E. Johnson, Allen R. Lewis, Richard L. Moody, Helen B. Moorhead, Russell R. Widner, Alma Thompson and Norma B. Naftaly

Cooperating Units: Connecticut Board of Education of the Blind
District of Columbia Department of Vocational Rehabilitation
Georgia Department of Education
Kansas Department of Social Welfare
Louisiana Department of Public Welfare
Massachusetts Department of Education
New Hampshire Department of Health and Welfare
New Jersey Commission for the Blind
New Mexico Department of Public Welfare
North Carolina Commission for the Blind
Oregon Commission for the Blind
Rhode Island Department of Social Welfare
South Dakota Service to the Blind and Visually Handicapped
Utah Department of Public Instruction
Vermont Department of Social Welfare
Virginia Commission for the Visually Handicapped

Man Years

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|---|---|
| Total: | 4 2/3 |
| Professional: | 3 8/20 |
| Others: | 1 6/20 |

Project Description:

Objectives: MRA is a voluntary association of States NINDB organized to maintain registers of persons with serious visual impairment. MRA defines blindness to include severe visual impairment. Each MRA State upholds membership standards and continues in membership as long as it upholds them. NINDB periodically evaluates each State's performance to insure that these standards are kept.

Each MRA State makes better blindness statistics available and stimulates research on blindness.

MRA:

1. Encourages a state to get all blind persons reported to make register reflect blindness prevalence and additions to reflect blindness incidence;

2. Seeks to improve blindness records, so blindness causes and blind population characteristics may be studied more easily;

3. Uses same blindness definition and same data record to make state data comparable for estimating blindness incidence and prevalence in entire United States.

Methods Employed: Each MRA State accepts MRA blindness definition which is "Visual acuity of 20/200 or less in better eye, with best correction; or visual acuity of more than 20/200 if visual field is restricted to 20 degrees." Best correction does not include devices which are not usable continuously or which improve visual acuity to more than 20/200 but restrict visual field to 20 degrees.

Each MRA state tries to register all MRA blind residents. They constitute its MRA Register. States may register other persons under a wider blindness definition, but only if they can be distinguished for statistical purposes. MRA Register is not limited to persons who have applied for welfare services; an MRA blind person may not be excluded from MRA register on basis of age, race, or any other factor.

MRA State tries to collect following information on each person. It routinely clarifies ambiguous reports, and obtains missing data. Required items must appear on register card (or its equivalent) for ready access. These items are essential minimum; however, State may collect additional pertinent information.

(a) When added to MRA register (month and year)
(b) First addition to MRA register or readdition
(c) Residence County (or its equivalent)
(d) Birth date (month, day, year)
(e) Sex
(f) Race (omitted if law prohibits recording it)
(g) When blinded age (month and year)
(h) Eye examination (month and year)
(i) Eye examiner discipline
(j) Visual acuity
(k) Blinding condition (site/type) and its cause (etiology)
(l) When removed from MRA register (month and year)
(m) Why removed from MRA register

Each MRA State corrects data elements in its register card when blind person is re-interviewed or eyes are re-examined. It immediately removes from MRA Register a person who died, moved out of State, or recovered vision beyond MRA blindness. Furthermore, it redetermines every registered person's residence and blindness status once a year. A person who received any services from blindness agency or has been contacted otherwise during year, is considered cleared if status has not changed since then. All other persons are contacted by mail inquiry, telephone call, or personal visit to registered person, relatives or neighbors, physician, or institution; a personal visit is made, when other methods fail. State completes these intensive efforts before year end, and removes from its MRA register all persons who have not been located. Person on MRA Register at year end is state resident and MRA blind.

A quality control program initiated in 1965 to get a variety of coders to classify blinding conditions and blind causes uniformly is now a continuing program. Each month, MRA states send coded physicians reports to NINDB where Medical Record Librarian verifies coding or supervises its verification; refers problem cases to NINDB staff ophthalmologist; and after review, returns reports to states with explanation of corrections.

MRA State prepares a deck of punched cards for each year, as NINDB specifies, and submits deck to NINDB by February 15 of following year.

Major Findings: MRA data for 1966 have been compiled and published in MRA's fifth report. Data on additions to MRA registers were published for 14 states. Population in registers at end of year were shown for 15 states. Major findings were as follows:

(a) A total of 61,037 persons were in MRA registers at end 1966. Persons with 20/200 visual acuity represented 20.8 percent of this total.

(b) Rates for additions generally remained low for ages under 45. Thereafter, rates increased markedly with age in each sex.

(c) The totally blind represented 5.2 percent of additions, and about 5.7 percent of them had visual acuity greater than 20/200 but with visual field restricted to 20 degrees. An additional 10.3 percent of additions had visual acuity of light perception or light projection.

(d) For all additions, three most frequent diagnoses were macular degeneration, cataract, and glaucoma (not congenital) which arbitrarily was coded to an etiology "unknown to science".

MRA's significance to bio-medical research and program of the Institute: MRA provides uniform data by state on visual acuity, blinding condition and its cause for additions to MRA registers by year. It also provides data on characteristics and attributes of persons in MRA register at end each year. MRA is only source for blindness data comparable by state and it is only source in which states use same blindness definition and extract data from same data elements. It is only source from which blindness data are available for such a large geographic area and such a large blind population. Incidence and prevalence data are needed by age, sex, race, visual acuity, and blinding condition and its cause for county, state, and national planning of blindness prevention and control, for providing services to the blind, allocation of funds for providing services to the blind, etc. Additions to MRA registers by year and population in them at end each year are needed for estimating annual blindness incidence and year end blindness prevalence by county, state, and United States total.

Proposed Course of Project: Improve data quality by getting unreported persons registered and removing persons from registers upon death, vision recovery, or departure from state; prepare an instruction manual explaining how to establish and operate an MRA register; prepare a manual for coding blinding conditions and their causes to serve as a supplement to present coding manual; improve annual statistical report, proceedings for annual conferences and brochure which explains MRA to the public; and admit new states into MRA to make MRA more representative of the entire country.

Honors and Awards: None

Publications:

Author: Section on Blindness Statistics, Office of Biometry, NINDB: Proceedings Sixth Annual Conference of Model Reporting Area for Blindness Statistics, 1967. U.S. Dept. of Health, Education, and Welfare, National Institutes of Health Publ. No. 1761, Washington, D. C., U.S. Government Printing Office, 1968

Author: Section on Blindness Statistics, Office of Biometry, NINDB: Statistics on Blindness in Model Reporting Area 1966. U.S. Dept. of Health, Education, and Welfare, National Institutes of Health Publ., Washington, D. C., U.S. Government Printing Office, 1968

Serial No. NDB (OD) - 63 B/BS 1137
1. Office of Biometry
2. Section on Blindness Statistics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: A Study of Awareness, Attitudes and Performance in Reporting Persons to Blindness Registers

Previous Serial Number: NDB (CF) - 63 B 1137

Principal Investigator: Paul H. Anderson

Other Investigators: Richard E. Onken (American Foundation for the Blind), Allen R. Lewis, and Alma D. Thompson

Cooperating Units: Blindness Register agencies in 29 states

Man Years

| | |
|---|---|
| Total | 11/20 |
| Professional: | 3/20 |
| Others: | 8/20 |

Project Description:

Objectives: Interview ophthalmologists, eye-ear-nose-and-throat specialists, and optometrists on awareness of, attitudes toward, and blindness reporting to state registers. Also, interview register agency staff, and study written materials and other communications that state register agencies send to ophthalmologists, EENT specialists and optometrists and forms and communications these agencies receive from them.

Methods Employed: Study populations included state register agency directors, staff members, and state consulting or supervisory eye specialists from 29 states, and ophthalmologists, EENT specialists and optometrists in these states. Directors of register agencies and staff members provided: Register uses, register law, register operation, services to blind persons, forms and communications to MRA register from sources that report blindness, and written materials and other communications from state blindness agencies, and to ophthalmologists, EENT specialists and optometrists, particularly educational campaigns to inform potential reporting sources and professional associations of desirability and need to report cases to register.

Ophthalmologists, EENT specialists and optometrists were selected from directories for interview. Respondents were located in urban areas with 50,000 or more population as of 4/1/60 to reduce interviewing costs. Letter sent to each person described study's purpose and included document of support from respondent's professional society. An appointment at respondent's convenience was arranged via a telephone call.

Interviews lasted one-half to an hour. Questionnaire included closed and open-ended questions which probed attitudes toward registers and reasons behind attitudes. Four separate questionnaires were used: one for agency personnel directors and staffs; a second for ophthalmologists who advise register agencies; a third for other ophthalmologists and EENT specialists, and a fourth for optometrists. Trained interviewers conducted interviews as directed by a commercial research firm under subcontract. Interviewing started June 1, 1965.

It was assumed that ophthalmologists and optometrists report blind persons to register agencies because they are aware of and have a positive attitude toward blindness registration, and do not report them due to lack of information and/or a negative attitude toward registration. This hypothesis was tested by grouping ophthalmologists, EENT specialists and optometrists as follows:

1. Those who report all blind persons to register agency.

2. Those who do not report because they are not aware of register and need to report all blindness cases, or are

3. Aware of register but unwilling to cooperate.

States will be compared on frequency and extent of reporting to ophthalmologists, EENT specialists and optometrists of need for reporting of all blindness cases, proportion of eye specialists and optometrists aware of register and information given by register agency, and proportion aware of register then will be related to state's effort to inform them of register.

Study will determine: Mandatory reporting effect on getting people reported, and attitude of potential reporting sources toward mandatory reporting in States with or without reporting laws.

It will: Determine if ophthalmologists or optometrists are more or less likely to report and why; evaluate state blindness agencies on orienting potential reporting sources as to registration benefits and will view results in relation to such orientation; will analyze attitudes to determine which lead to reporting and which lead to nonreporting; develop codes and analyze contents before data collection; since both closed and open-ended questions will be asked, tabulate collected data by computer; and will analyze attitudes to register agency, welfare agencies in general, to blind persons, etc.

Major Findings: Data have been collected but not analyzed.

Significance to Bio-medical Research and Program of the Institute: This study will get more blind persons reported to state register, by revealing how and why attitudes hinder or encourage reporting. Therefore, analyzed research findings will be translated into recommendations for implementation.

The study report and its evaluation should help states to get a higher proportion of the blind reported to MRA registers. Section on Blindness Statistics, Office of Biometry, NINDB should improve its consultative services to MRA states by implementing this survey's findings.

This study will measure and evaluate register agency efforts to encourage blindness reporting to state registers. It will relate communications they directed to eye specialists and optometrists with their awareness of these communications. If these professionals are not aware of communications, register agencies may be advised to improve communication practices based on practices of more successful States and practices MRA adopted. If, reporting sources are aware of register and need to report all blindness cases but do not do so, the nonreporting reasons will be determined. If nonreporting results from objection to procedures in registration, then register agency may be advised to change such procedures, or to inform reporting sources of need for these procedures.

Proposed Course of Project: Prepare tables, analyze data, and publish findings.

Honors and Awards: None

Publications: None

Serial No. NDB (OD) - 64 B/BS 1188
1. Office of Biometry
2. Section on Blindness Statistics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Study of Blindness in Egypt

Previous Serial Number: NDB (CF) - 64 B 1188

Principal Investigators: Hyman Goldstein and Paul H. Anderson

Other Investigator: Dr. Mohyi-Eldin Said,
University of Alexandria, Egypt

Cooperating Units: University of Alexandria, Alexandria, Egypt

Man Years

| | |
|---|---|
| Total: | 1/10 |
| Professional: | 1/20 |
| Others: | 1/20 |

Project Description:

Objectives: To ascertain feasibility of developing a blindness register from a pilot study in Egypt for producing blindness statistics for blindness prevention and control; determine how Model Reporting Area (MRA) for Blindness Statistics in United States must be modified for application in Egypt to permit production of comparable statistics; and document problems and their solutions in conducting such studies in developing countries.

Methods Employed: Study is conducted in two urban areas (approximately 125,000 persons) and in 23 rural villages (approximately 125,000 persons). Study has three phases. In Phase I which has been completed a random sample of about 1,000 households (about 5,000 persons) was drawn from urban study areas for house-to-house vision testing. Same size sample was drawn from rural study areas. With an estimated blindness prevalence rate of 15 per 1,000 population (using a blindness definition comparable to MRA's), survey was expected to yield about 75 blind persons in urban areas and same number in rural ones in this Phase. Actually, using MRA blindness definition, prevalence in urban area was 14 per 1,000 and 45 per 1,000 in rural areas. The estimated prevalence rate in the United States is about 2 per 1,000. About one and one-half years later Phase II reexamined persons in original sample of 10,000 persons still resident in study areas to determine new blindness cases. In both phases, the examined population found not to be blind, is the control group. Phase II examined every resident in study areas by persuading such residents to come to health centers voluntarily for a vision examination. This established a blindness register. Assuming that

its prevalence rate of 31 per 1,000 population found in Phase I, obtains in Phase II, approximately 7,750 persons should be blind in this Phase.

In Phase I, persons in control group were those not blind, or 10,675 of 11,023 actually examined. The same is true of Phase III. In Phase II which established a blindness register, approximately 7,750 persons were cases and 242,250 were controls.

Information collected included:

(a) Cases and controls: Birth date, birth place, sex, nationality, race, marital status, education, occupation, distance vision both uncorrected (or with present corrections) and with best correction for each eye, refractive power of lenses, peripheral visual field where better eye with best correction has visual acuity greater than 6/60.

(b) Cases only: Probable age when vision was severely impaired, history of severe ocular infections, operations, etc., with age at occurrence, blood relationship, if any, between parents, history of similar ocular conditions in blood relatives, near vision both uncorrected (or with present correction) and with best correction for each eye, intraocular pressure, cause of severe visual impairment (site and etiology), prognosis and recommendations for treatment, if any.

Total and cause-specific incidence and prevalence rates will be compared for various age, sex, urban and rural, and other groups. Distribution of demographic variables in cases will be compared with their distributions in control groups.

Major Findings: Data are still being collected. Study will continue until September 1968.

Significance to Bio-medical Research and Program of the Institute: This is first attempt to apply United States data collection procedures (MRA) to a developing country to determine how these procedures must be modified for application in a developing country to permit production of comparable statistics. Use of such a "tool", in cooperation with scientific sampling in underdeveloped countries has tremendous potential in derivation of comparable international statistics of blindness. Since Egypt's blindness prevalence is one of world's highest, development of comparable statistics for that country may provide valuable clues to role of environment and other factors in blindness etiology. Specific information on age, sex and other demographic variables in blind population will help pinpoint high risk groups who need to be screened for early signs of eye disorder.

Proposed Course of Project: Collect, process, analyze and interpret data and publish reports.

Honors and Awards: None

Publications: None

Serial No. NDB (OD) - 67 B/BS 1323
1. Office of Biometry
2. Section on Blindness Statistics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Further Analysis of Perinatal Factors and Strabismus

Previous Serial Number: NDB (CF) - 67 B 1323

Principal Investigator: Russell R. Widner

Other Investigators: J. Theodore Schwartz, Epidemiology Branch, C&FR, NINDB, and Hyman Goldstein

Cooperating Units: Section on Ophthalmic Field and Developmental Research
Epidemiology Branch, C&FR, NINDB
Division of Research, Children's Bureau, Department of Health, Education and Welfare
Wilmer Eye Clinic of Johns Hopkins Hospital, Baltimore, Maryland
Greater Baltimore Medical Center, Baltimore, Maryland
Baltimore City Eye-Ear-Nose-and-Throat Hospital, Baltimore, Maryland
University of Maryland Hospitals, Baltimore, Maryland

Man Years

| | |
|---|---|
| Total: | 1/6 |
| Professional: | 1/6 |
| Others: | |

Project Description:

Objectives: To investigate relationship of birth-weight to occurrence of accommodative esotropia, non-accommodative esotropia, and exotropia.

Methods Employed: A retrospective investigation was made of the 400 children which comprised the case study group for study Serial No. NDB (CF) - 63 B 1134 and which was reported (Goldstein, Henderson, Goldberg, Benitez, and Hawkins: "Perinatal Factors Associated with Strabismus in Negro Children," American Journal of Public Health 57: 217-228, February, 1967). Cases were grouped into three groups: Non-accommodative esotropia, accommodative esotropia, and exotropia. Information included details essential to classification of strabismus cases as well as certain sensory phenomena in details of treatment. The three groups were compared with their matched controls regarding birthweight.

Major Findings: The case reviews are completed. The statistical analysis is near completion. The statistical analysis is expected to be disappointing because none of the groups showed a highly significant relationship of the occurrence of strabismus in children of low birthweight.

Significance to Bio-medical Research and Program of the Institute: As stated previously Hyman Goldstein, et al, showed a highly significant relationship between birthweight and strabismus. Unfortunately, an in-depth review of the records reveals that many children suffered from strabismus which probably was secondary to other ocular conditions. These ocular conditions included congenital cataract, congenital glaucoma, congenital chorioretinitis, plane syndrome, ptosis, marked anisometropia, and various cranial nerve paralysis. Many cases possibly had low birthweight; therefore, the remaining cases for this study did not confirm the results of the previous study.

Proposed Course: If statistical results prove to be disappointing as expected, study will be terminated.

Honors and Awards: None

Publications: None

ANNUAL REPORT

July 1, 1967 through June 30, 1968

Direct Training

National Institute of Neurological Diseases and Blindness
National Institutes of Health

Funds under this activity provide for the support of the Institute's training programs for professional, technical, administrative, and clerical personnel, including job-related training at both government and non-government facilities.

Commissioned Officers from the Intramural, Extramural, and Collaborative and Field Research program areas are receiving basic training at the State University of New York, Syracuse; at the Mayo Clinic, Rochester; and at Grady Hospital in Atlanta.

Training in neurology was supported also for Commissioned Officers of the Division of Hospitals at Southwestern Medical School, Dallas and University of Washington, Seattle and in otolaryngology at Washington University, St. Louis.

Short-term courses are also being supported as funds are available.

Table of Organization

Intramural Research

National Institute of Neurological Diseases and Blindness
(Personnel on hand May 1968)

Office of Associate Director:

Director of Intramural Research - Henry G. Wagner, M.D.
Clinical Director - Maitland Baldwin, M.D., M.B., F.R.C.S.
Administrative Officer - Glenn E. Hammond
Mathematician - Rosalind B. Marimont
Administrative Officer - Elizabeth W. Snowden
Administrative Officer - J. Loring Jenkins
Photographer - Wesley Pearson Jr.
Senior Surgeon - Frederick J. Samaha, M.D.
Surgeon - Gerald D. Fischbach, M.D.
Surgeon - Robert E. Burke, M.D.
Surgeon - William I. Rosenblum, M.D.
Scientist - Donald R. Humphrey, Ph.D.
Surgeon - Martin A. Hatcher, M.D.
Surgeon - Lawrence S. Zivin, M.D.
SA Surgeon - Alan L. Norton, M.D.
Electronic Technician - Alvin K. Kestner
Special Assistant - Nancy F. Nusbaum
Secretary (Stenography) - Maxine O. Reynolds
Travel Assistant - Ida M. Isaacson
Photographer - Jerry M. Ellis
Procurement Clerk - Doris R. Perry
Secretary (Stenography) - Margret L. Shipley
Biological Laboratory Technician - George R. Duvall
Clerk-Stenographer - Virginia L. Gray
Budget Clerk - May I. Ferrari
Biological Laboratory Technician - Adrian P. Loftis

Section on Technical Development:

Computer Programmer - William H. Sheriff Jr.
SA San E - Howard M. Seidband

Medical Neurology Branch:

Chief - W. King Engel, M.D.
Associate Neurologist - Dale E. McFarlin, M.D.
SR Surgeon - Michael H. Brooke, M.D.
Surgeon - Gregory O. Walsh, M.D.
Surgeon - David E. Pleasure, M.D.
Surgeon - Frederic Q. Vroom, M.D.
Surgeon - Paul W. Hathaway, M.D.
Surgeon - David S. Dahl, M.D.
Surgeon - William H. Olson Jr., M.D.
Surgeon - Darryl C. De Vivo, M.D.
Biological Laboratory Technician - Guy G. Cunningham
Chemist - Mary E. Collins
Biological Laboratory Technician - Donald W. Bishop
Medical Technologist - Kathleen C. Mishler
Clerk-Dictating Machine Transcriber - Patricia D. Williams
Clerk-Dictating Machine Transcriber - Lucy L. Riley
Clerk-Dictating Machine Transcriber - Gertrude E. Wright
Medical Technician - Pauline R. Middleton
Laboratory Worker - Matthew P. Meadows

Section on Biophysical Applications:

Surgeon - Robert C. Griggs, M.D.
Secretary (Stenography) - Vernita Bergmeyer
Physiologist - Carolyn E. Derrer

Section on Neuroradiology:

Research Medical Officer - Giovanni Di Chiro, M.D.
Clerk-Stenographer - Janice A. Dawn

Section on Clinical Applied Pharmacology:

Supervisory Research Pharmacologist - Richard L. Irwin, Ph.D.
Research Physiologist - Jay B. Wells, Ph.D.
Biologist - Katherine L. Oliver
Secretary (Dictating Machine Transcriber) - Emma P. Dick

Surgical Neurology Branch:

Associate Neurosurgeon - John M. Van Buren, M.D.
Medical Officer - Choh-luh Li, M.D.
Medical Officer - Ayub K. Ommaya, M.D.
Surgeon - John F. Davison, M.D.
Surgeon - Robert A. Ratcheson, M.D.
Surgeon - Nicholas E. Grivas, M.D.
Surgeon - Duane B. Gainsburg, M.D.
Surgeon - Edward C. Tarlov, M.D.

Surgical Neurology Branch (cont'd.)

Biologist - Rosemary C. Borke
Biologist - Levon O. Parker
Supervisory Operating Room Nurse - Frances D.S. Lamberti, R.N.
Supervisory Operating Room Nurse - Mildred L. Haddox, R.N.
Operating Room Nurse - Delta H. Trickett, R.N.
Operating Room Nurse - E. Dolores Blessing, R.N.
Operating Room Nurse - Barbara L. Edmonds, R.N.
Clerk (Dictating Machine Transcriber) - Helen M. Andregg
Clerk-Stenographer - Gertrude C. D'Addio
Clerk (Typing) - Celeste L. Sewell
Medical Aid - Morris M. Williams
Medical Aid - M. Arthur Banks
Clerk-Stenographer - Cheryl R. Parks
Secretary (Stenography) - Michele Y. Shevitz
Clerk-Stenographer - Dawn M. Broadhurst
Medical Aid - Howard F. Stewart Jr.
Medical Aid - Octavie W. Jacobs
Biologist - Kenneth E. Parker
Biological Aid - Vernon G. Mastin Jr.

Section on Primate Neurology:

Biologist - Kenneth Rich
Biological Laboratory Technician - Norman E. Mills
Medical Aid - Charles E. Sartor
Biological Aid - Leo Jacobs

Section on Neuroanesthesiology:

Visiting Scientist - Yushi Kondo, M.D.
Biological Laboratory Technician - Calvin S. Hawkins

Section on Clinical Psychology:

Supervisory Research Psychologist - Herbert C. Lansdell, Ph.D.
Research Psychologist - Paul Fedio Jr., Ph.D.
Secretary (Typing) - Lillian L. Wease

Section on Clinical Neuropathology:

Visiting Scientist - Barbro B. Johansson, M.D.

Section on Child Neurology:

Medical Officer - Anatole Dekaban, M.D.
Surgeon - Joseph H. Zelson, M.D.
Research Chemist - Dennis F. Cain, Ph.D.
Research Chemist - George Constantopoulos, Ph.D.

Section on Child Neurology (cont'd.)

Secretary (Stenography) - Linda M. Ingram
Medical Technician - Jan K. Steusing
Chemist - Diana E. Mizel

Electroencephalography Branch:

Chief - Cosimo Ajmone Marsan, M.D.
SR Surgeon - Andrew J. Gabor, Ph.D., M.D.
Surgeon - Russell W. Hardy Jr., M.D.
EEG Technician (Supervisory) - Rosemarie Schilling
EEG Technician - Audrey T. Rinaldi
Secretary (Dictating Machine Transcriber) - Elizabeth A. Lozada
Clerk (Dictating Machine Transcriber) - Barbara G. Nachman
Biological Aid - Myrtle Ann Jones
EEG Technician - Anne M. Moyer

Section on Clinical Neurophysiology:

Surgeon - Don C. Bienfang, M.D.
Biologist - John H. Jones

Ophthalmology Branch:

Chief - Ludwig von Sallmann, M.D.
Medical Officer - Vernon G. Wong, M.D.
Surgeon - Daniel M. Albert, M.D.
Surgeon - Steven M. Podos, M.D.
Surgeon - Thomas F. Minas, M.D.
Surgeon - Eliot L. Berson, M.D.
Surgeon - Richard S. Smith, M.D.
Chemist - Patricia A. Grimes
Medical Technician - Eleanor M. Collins
Ophthalmology Technician - Gerald S. Hoover
Biologist - Richard R. Anderson
Secretary (Stenography) - Rita U. Durant
Clerk (Typing) - Harriett Murphy
Clerk-Stenographer - Dee Ann Lodge
Clerk(Typing) - Ethel F. Butts
Biologist - Mercedes M. Saur
Biological Laboratory Technician - Luther R. Dowell Jr.
Clerk-Typist - Nancy M. Grams

Section on Ophthalmology Physiology:

SR Surgeon - Peter Gouras, M.D.
Physicist (Optics) - Ralph D. Gunkel, O.D.
Electronic Technician - James L. Jones
Biologist - Mary J. Hoff

Section on Ophthalmology Chemistry:

Research Chemist - Marc S. Lewis, Ph.D.
Staff Fellow - Ralph J. Helmsen, Ph.D.
Research Chemist - Hitoshi Shichi, Ph.D.

Section on Ophthalmic Metabolism:

Research Chemist - Paul J. O'Brien, Ph.D.
Research Chemist - J. J. Bungenberg de Jong, Ph.D.
Chemist - Consuelo G. Muellenberg

Section on Cell Biology:

Research Biologist - Arnaldo Lasansky, M.D.
Biological Laboratory Technician - Julia Matthews

Section on Ophthalmology Pharmacology:

Supervisory Pharmacologist - Frank J. Macri, Ph.D.
Biological Laboratory Technician - Joseph G. Brown

Laboratory of Neuropathology & Neuroanatomical Sciences:

Chief - Igor Klatzo, M.D.
SA Surgeon - Andrew A. Zalewski, M.D.
Photographer - Edwin H. Moodhe
Secretary (Stenography) - Vivian K. Arnold
Biological Laboratory Technician - Willie Perkins
Clerk (Typing) - Hilda M. Malcolm
Secretary (Typing) - Marie H. Dickerson
Biological Laboratory Technician - Albert V. Cantu
Biological Laboratory Technician - Melvin H. Carroll

Section on Experimental Neurology:

Medical Director - Lloyd Guth, M.D.
Physiologist - Herbert Yellin, Ph.D.
Biological Laboratory Technician - Janina D. Ziemnowicz
Biologist - William C. Brown
Biologist - Phyllis K. Watson

Section on Neurocytology:

Research Biologist - Keith C. Richardson, B.Sc., M.Sc.
Research Physiologist - Milton W. Brightman, Ph.D.
Visiting Scientist - Yngve O. Olsson, M.D.
Surgeon - Arthur I. Geltzer, M.D.
Surgeon - Charles D. Roth, M.D.
Biological Laboratory Technician - Clara E. Mowry

Section on Functional Neuroanatomy:

Head, Section on Functional Neuroanatomy - Grant L. Rasmussen, Ph.D.
Medical Officer - Thomas S. Reese, M.D.
Surgeon - Donald M. McDonald, M.D.
Biological Laboratory Technician - Frank D. Nolan
Biologist - Helen J. Osborne
Medical Technologist - Edna P. McCrane

Section on Comparative Neuropathology:

Supervisory Medical Technician - Joseph T. Walker, Jr.
Biological Laboratory Technician - Ernestine G. Dye
Medical Technician - Thelma R. Fletcher

Section on Experimental Neuropathology:

Medical Officer - Jan Cammermeyer, M.D.
Biologist - Iris Mercado
Biological Laboratory Technician - Margaret G. Johnson
Editorial Assistant - Jane T. Phelps
Biological Aid - Sophia Grabinski
Laboratory Worker - Kenneth M. Oglesbee

Laboratory of Biophysics:

Supervisory Research Physiologist - Robert E Taylor, Ph.D.
Senior Research Biophysicist - Kenneth S. Cole, Ph.D., D.Sc.
Secretary (Stenography) - Grace C. Ream
Clerk-Stenographer - Constance S. Till

Section on Instrumentation:

Electronic Engineer - Leonard Binstock
Electronic Technician - Herbert A. Walters

Section on Mathematical Biophysics:

Research Physicist - Richard FitzHugh, Ph.D.
Mathematician - John Shaw
Research Physicist - Harold Lecar, Ph.D.

Section on Membrane Biophysics:

Research Physicist - Gerald M. Ehrenstein, Ph.D.

Section on Cellular Biophysics:

Research Physiologist - Daniel L. Gilbert, Ph.D.
SR Surgeon - Irving M. Stillman, M.D.
Biologist - Allen D. Spencer Jr.

Laboratory of Neurochemistry:

Chief - Donald B. Tower, M.D., Ph.D.
Visiting Fellow - Janice Skidmore, Ph.D.
Secretary (Stenography) - Shirley Ann Burdette
Clerk-Stenographer - Myrna S. Shenkler

Section on Amino Acid Chemistry:

SR Surgeon - Robert S. Bourke, M.D.
Chemist - Oscar M. Young

Section on Physiology and Metabolism:

Research Chemist - Eberhard G. Trams, Ph.D.
Chemist - Carl J. Lauter

Section on Enzyme Chemistry:

Research Chemist - R. Wayne Albers, Ph.D.
Surgeon - George J. Siegel, M.D.
Chemist - George J. Koval
Physical Science Aid - Eunice L. Summers

Section on Lipid Chemistry:

Head, Section on Lipid Chemistry - Roscoe O. Brady, Jr., M.D.
Research Chemist - Andrew E. Gal, Ph.D.
Research Chemist - Julian N. Kanfer, Ph.D.
Chemist - Roy M. Bradley
Chemist - Jane M. Quirk
Surgeon - Roger A. Snyder, M.D.
SA Surgeon - Neal J. Weinreb, M.D.
Biological Aid - Alexander B. Wheaton
Biological Laboratory Technician - Frank J. Fash

Laboratory of Neurophysiology:

Chief - Michelangelo Fuortes, M.D.
Research Medical Officer - Phillip G. Nelson, M.D., Ph.D.
Medical Officer - Thomas G. Smith Jr., M.D.
Physicist - Margaret W. Chapman
SR Surgeon - Norman Robbins, M.D.
SA Surgeon - William K. Stell, M.D.
Surgeon - John J. Hubbard Jr., M.D.
Biological Laboratory Technician - William L. Beane
Secretary (Dictating Machine Transcriber) - June M. Picone
Laboratory Worker Leader - Clifford A. Seay
Laboratory Worker - Harold E. Smith
Visiting Associate - Peter Winter, Ph.D.

Laboratory of Neural Control:

Chief - Karl Frank, Ph.D.
SA Surgeon - David J. Mishelevich, M.D.
Secretary (Stenography) - Frances I. Cryan

Laboratory of Molecular Biology:

Chief - Ernst Freese, Ph.D.
Research Chemist - Robert A. Lazzarini, Ph.D.
Research Biologist - Elisabeth G.M. Freese, Ph.D.
Research Medical Officer - L. Robert Berberich, M.D.
Research Chemist - Hans-Jurgen Rhaese, Ph.D.
Surgeon - Charles M. Cashel, M.D.
SA Surgeon - Robert M. Winslow, M.D.
Chemist - Stephen S. Sklarow
Biological Laboratory Technician - Uta Fortnagel
Chemist - Enid M. Galliers
Secretary (Stenography) - Mary W. Taylor
Mathematics Technician - Naomi K. Boetker
Clerk-Stenographer - Crystal L. Bixler
Biological Laboratory Technician - Blanche E. Lewis
Visiting Fellow - Kiyoi Nakata, Ph.D.
Chemist - Barbara J. Kalbacher

Laboratory of Perinatal Physiology: (Puerto Rico)

Chief - Ronald E. Myers, Ph.D., M.D.
Scientist - Sven O.E. Ebbesson, Ph.D.
Surgeon - Ronald A. Cyrulnik, M.D.
Surgeon - James R. Miller, M.D.
Surgeon - Alfred W. Brann, M.D.
SA Surgeon - Chester P. Swett Jr., M.D.
SA Surgeon - Harvey M. Shapiro, M.D.
Research Chemist - Americo Rivera Jr., Ph.D.
Research Psychologist - Shun-ichi Yamaguchi, Ph.D.
Research Psychologist - Margaret Varley, Ph.D.
Administrative Officer - C. Torres Colondres
Administrative Assistant - Orlando Nieves
Equipment Specialist - George M. Dold
Research Psychologist - Bruno Kohn, Ph.D.
Research Psychologist - Elizabeth A. Missakian, Ph.D.
Librarian - Jorge Rivera-Ruiz
Photographer - Pedro Sola-Amadeo
Biological Laboratory Technician - Joaquin M. Segarra
Chemist - Carmen L. Freixas
Biological Laboratory Technician - Maria L. Alvarado de Yamaguchi
Biologist - Rafael Rivera
Biological Laboratory Technician - Luis C. Gonzalez
Biologist - Maria A. Rivera de Rivera
Biologist - Luis Raul del Rio

Laboratory of Perinatal Physiology (Puerto Rico-Cont'd)

Medical Technician - Maria D. Lopez
Personnel Assistant - Ruby C. Gittens
Secretary (Stenography) - Mary Alice Rollings
Clerk - Sara L. Mendez
Biological Laboratory Technician - Calixto Jimenez-Lugo
Supply Clerk - Carlos M. Rosario-Rivera
Biological Laboratory Technician - Carmelo Pagan-Blanchero
Secretary (Stenography) - Teresa A. Del Rio
Medical Technician - Luis Rivera-Baez
Secretary (Stenography) - Paulina T. Papp
Clerk - Carmen O. De Garcia
Biological Laboratory Technician - Luz Delia Gonzalez Mercado
Biological Laboratory Technician - Jose E. Oliver
Biological Laboratory Technician - William Rodriguez
Clerk-Typist - Hilda M. Currier
Biological Aid - Gladys Diaz de Torres
Photographer - Luis Villafane-Santiago
Clerk-Typist - Emelie I. Ortiz
Biological Aid - Rafael Rivera-Correa
Telephone Operator - Luisa E.V. Sneed
Clerk-Stenographer - Estebania P. Perez
Guard - Manuel Solla Hernandez
Messenger - Alfredo Perez
Facilities & Equipment Mechanic - Manuel Maldonado-Rosa
Carpenter - Hipolito Gonzalez
Carpenter - Domisiano Melendez
Animal Caretaker - Demetrio Melendez
Animal Caretaker - Policarpio Quinones
Animal Caretaker - Emilio Tolentino
Animal Caretaker - Gregorio Figueroa
Animal Caretaker - Inocencio Robledo
Animal Caretaker - Manuel Rosado-Rosario
Laborer - Cayetano Guzman
Animal Caretaker - Eliezer Melendez-Robledo
Animal Caretaker - Efrain Melendez-Robledo
Animal Caretaker - Bernardino Santana
Laborer - Aristides Quinones
Laborer - Romualdo Rivera-Rivera

Laboratory of Perinatal Physiology - Puerto Rico - Islands

SA Veterinarian - Ronald F. Di Giacomo, DVM
Research Biologist - Halsey M. Marsden, Ph.D.
Scientist - Stephen H. Vessey, Ph.D.
Construction & Maintenance Superintendent - Carlos R. Nagel
Chemist - Jose Martinez de Jesus
Clerk-Stenographer - Susan J. O'Higgins
Biological Aid - Victor A. Bracero-Pagan
Carpenter - Jacinto Salvador Rosado

Laboratory of Perinatal Physiology - Puerto Rico - Islands (cont'd.)

Carpenter - Jose A. Flores
Biological Aid - Angel Figueroa-Velez
Animal Caretaker - Israel Cordero
Animal Caretaker - Francisco Ruiz Rivera
Carpenter - Heriberto Moreno
Animal Caretaker - Luis I. Hernandez
Animal Caretaker - Hector L. Vazquez
Laborer - Alejandro Ilarraza
Animal Caretaker - Francisco Acevedo Soto
Clerk-Typist - Miguel Bernard-Melendez
Laborer - Maximino Reyes Rivera
Laborer - Pedro A. Robles

ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR
OF THE
NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

July 1, 1967 through June 30, 1968

Several changes have been made during the past year in the administrative organization of the Intramural Program.

A new Laboratory of Neuropathology and Neuroanatomical Sciences has been created to consolidate the research, staff and facilities formerly identified with the separate Laboratories of Neuropathology and Neuroanatomical Sciences. The former Laboratories always had close interests, worked in proximity, inter-communicated well and used common technology. The consolidation will offer new channels of mutual support and should work to the benefit of a total program.

Also, a new Laboratory of Neural Control was established. The mission of this Laboratory is to conduct research leading to the development of neural control of external devices through the use of central or peripheral nervous effectors. The program will be a broad one to look also for methods of inward information transfer; external control of the nervous system. It will also conduct research leading to the development of neural modeling for the purpose of studying mechanisms of information processing in the central nervous system.

A third change has been the establishment of a Laboratory of Neurophysiology, administratively completely within NINDB. The Spinal Cord Section of the previous joint NINDB-NIMH Laboratory, which represented the NINDB interest, has been transferred to form the nucleus of the new Laboratory. The new Laboratory will execute a broad research program primarily directed toward developing a better understanding of sensory mechanisms, nerve cells and synapses, and sensory and motor systems.

Completion of the new NINDB-NIMH Laboratory Building has been further delayed as a consequence of certain constructional problems. Actual movement of laboratories into the new Building is now expected to start sometime in the early part of the new Fiscal Year.

Turnover of total personnel for FY 68 reached 40 resignations to 45 recruitments. This has resulted in a net increase in on-board strength from 316 to 321. The quality and number of new recruits

remained at satisfactory levels. Turnover of professional GS-11 and above has shown an improvement in acquisitions (32) to resignations (24). This high turnover is due largely to Public Health Service Officers completing obligated 2-year tours.

The number of active projects during the past year was 188 with 23 terminated for various reasons, 29 completed and 62 new projects established.

The Intramural Program has continued to grow and make significant progress in accomplishing its mission. Highlighted here are illustrative facets. Greater detail may be found in the reports of the Laboratory and Branch Chiefs.

Basic research for the acquisition of new knowledge and understanding of neurobiological processes continues to be emphasized. Further inquiry has been directed toward the problem of how light produces electrical changes in the photoreceptor cells of the eye. Considerable attention has been given to the question of whether nerves exert a "trophic" effect on the structures they innervate. Can we explain the difference between fast and slow twitch muscles as due to some specific "trophic" effect of motoneurones. Our research seems to show that the nerves do indeed exert some sort of control over the properties of the muscle fibers, but because of the complexity of factors operating in the situation, such as in use or disuse of the muscle much more work is needed to sort out the nature of the effect. The problem is an important one, however, for the success of therapy for muscles and nerves which are injured or diseased are particularly dependent on this type of knowledge.

Significant progress has been gained in the understanding of the genesis of the electroencephalogram. Now it is possible to correlate experimentally observed EEG potentials with those predicted by a mathematical theory of electrotonic and volume conductors. Possible differences in the mechanism of the electrogenic effects by different pharmacological agents on cortical neurons is being investigated and will be reported on in the near future; the incidence and significance of epileptiform patterns in EEG records in over 6,000 subjects, is also being collected.

The use of anti-metabolites like methotrexate and cytoxan has been found to be beneficial in the management of some types of uveitis of the eye even after steroid treatment has become ineffective. Unfortunately, relapse appears not to be prevented and some forms of uveitis such as sympathetic ophthalmia do not benefit reliably. Of considerable interest is our finding that the beneficial value of immunosuppressive drugs in the treatment of uveitis like sympathetic ophthalmia is due more to its anti-inflammatory properties rather

than the immuno-suppressive action. Another important event is the discovery of a new method for extracting rhodopsin from the retina. This extract has the highest spectral purity reported and seems to be a single homogenous component. It may well prove to be a major assist to workers on the chemistry of visual processes. Further progress can also be reported on the characterization of lens protein, control processes in synthesis of glycoproteins and the pharmacology of new drugs used in the treatment of glaucoma. An unusual ocular abnormality was discovered in neonatal monkeys subjected to artificial interruption of blood flow to the head. This insult resulted in a type of cystic degeneration of the retina which showed remarkable resemblance to certain congenital abnormalities occasionally seen in the human eye. This is a segment of our major area of study of the influence of noxious events during the perinatal period. Fetal asphyxia is an important focus of effort. Another experimental thrust is directed towards understanding the curious protection provided by barbiturates against brain damage in asphyxia. Another related study now substantially complete follows up some early indications that the brain of the adult monkey can survive ischemia up to 20 minutes without serious damage. High tolerance of the fetal brain to asphyxia had previously been established. The completed study confirms this finding in adults as well.

Our further experience with intrathecal chemotherapy using methotrexate has disclosed that it is not a useful agent against glioma. At normal body temperatures, the blood brain barrier effectively prevents drugs reaching the brain substance itself. Several approaches to the problem are being carried out simultaneously. One approach is intrathecal injection of drugs. Another is whether the radical increase in permeability of the brain to drugs at low temperature can be of advantage. In this avenue of approach, we have been gratified by considerable success in the treatment of intractable epilepsy. Studies are in progress with a variety of drugs in a number of conditions, both on patients and in animal research where many of the basic principles must be worked out for optimum performance.

Our studies on cerebral trauma are being continued. These have focused on the role of whiplash injury in the brain. High rates of rotational acceleration can reliably cause unconsciousness in experimental animals without other evidence of impact injury. A model theoretical formulation is being checked out with different sized animals which, if found to fit well, the data will allow extrapolation to man. The importance of this point is that a good animal model will then exist.

A new classification of biochemical abnormalities in the myopathies has been prepared. A rather dramatic finding has been that ACTH therapy has provided definite improvement in atypical childhood myasthenia gravis. We have had excellent clinical response with Azothioprine in inflammatory myopathy and Acelozolamide has been found to be the best prophylactic agent in the treatment of periodic paralysis.

In collaboration with the Department of Diagnostic Radiology, CC, selective arteriography of the spinal cord has been developed to a high state of precision and procedures and instrumentation have been developed which make it possible to do brain scanning with multiple radioisotopes.

The momentum achieved in our prior success with finding the nature of the metabolic defects in certain of the hereditary lipid diseases like Fabry's, Gaucher and Niemann-Pick has been extended with the discovery of the nature of the sphingolipid defect in the disease metachromatic leukodystrophy. Our initial studies on the use of Isethionate in reducing cerebral edema has shown sufficient promise in animal research that this work will be explored for possible application in man.

A new type of lipid has been identified in cells which appears to act as a trans-membrane carrier molecule for long chain fatty acids. The aminoacid methionine has been found to play a key role in lipoprotein synthesis and may be an essential component of the protein variety of lipoproteins.

We have continued to support vigorously our research on the control mechanisms relating DNA to RNA synthesis. These studies are important because so many diseases arise from molecular disorders of cell metabolism, which seem to arise in a fault in replication of appropriate enzymes. Particular highlights in this research are that the growth rates of RNA have been measured and a new general repressor nucleotide for RNA synthesis has been discovered. Another study has found that the citric acid cycle is not necessary for vegetative growth in Bacillus Subtilis, the experimental model for this research, but is essential for sporulation.

Our long-range study of the specific molecular mechanisms of nerve conduction has focused on ion movements in squid giant axones. This axone is unique in that the internal as well as external media can be altered along specifically desired lines. Squid is generally available in only a few remote localities which must be used since no practical means of transporting axones in good condition has been developed. Preliminary studies of the giant axone of the marine worm

Mixicola Infundibulae have shown great promise that it may be an equivalent substitute. Since it can be shipped in good condition at all times of the year, it will have many advantages to accelerate these studies.

Attention has been directed to the microstructure of the autonomic nervous system. In particular, histochemical techniques have shown distinct morphologic detail of the innervation of the sphincter and dilator muscles of the iris, and a new type of degeneration of axonal endings in the auditory pathways has been found. The cytologic characteristics in microglia in animals raised germ free have been compared to these cells in conventional normal animals. Since these cells are believed to play a phagocytic role in the nervous system, this study is expected to clarify their role in central nervous system disease.

Finally, our program in primate ecology continues to be productive. Recent work has concentrated on vocal communications, determinants of dominance, daytime sleep patterns, social behavior of castrates, agonistic behavior, inhibition of son-mother matings, seasonal breeding. New work is in progress on the social structure and significance of kinship ties and the matriarchal structure of monkey society. Important significance is attached to the differences being observed in social behavior between wild-reared and cage-reared monkeys. The experience and learning acquired in infancy and childhood of social structure and behavior is nowhere more clearly obvious than in these comparisons. Social gestures, grooming, mating, etc., are vastly different in the two situations. Opportunities to further this important field of study are open and will be sought. Behavioral studies seem to show that the monkey has no clear-cut volitational control of its vocalization. This is surprising since the sounds uttered by monkeys are extremely complex and varied. The facts, however, suggest that, while the emitted sound clearly reflects the emotional state of the animal, it cannot be trained to produce a particular sound as a conditioned response.

It is believed that future progress will continue at an accelerated pace and significant contributions to the mission will increase.

Serial No. NDB(1)-66/OAD 1460
1. Office of Associate Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Survey of feasibility of visible speech display as an aid for the deaf.

Previous Serial Number: Same

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Man Years:
Total: .25
Professional: .25
Other: 0

Project Description:

Objectives: To study the feasibility of using a visual display of speech as an aid in speech training of the deaf.

Methods:
1. Review of status of current visual displays through the literature and consultations.
2. Review of status of research in allied field, such as machine decoding of speech.
3. Analysis of problems encountered to date.

Major Findings:

Results and visual displays

1. Considerable work has been done in the use of visual displays of various speech parameters. The displays may be divided into two general classes. The first, which might be called total displays, show in some form the complete acoustic signal, such as the Bell Visible Speech Translator and by Kopp and Kopp at Wayne State University, and its modified form used by Stark at Johns Hopkins University. The second, which might be called partial displays, show some particular feature of the acoustic signal, such as fundamental pitch, formant structure of vowels, consonsnt and vowel timing, the quality of being voiced or voiceless, stress, and others.

Results of all this work have been encouraging in some ways, and discouraging in others. The efficacy of a visual feedback loop to guide muscular behavior has been demonstrated beyond question. Those partial displays which give clear unambiguous patterns, in which success or failure at

matching can be easily determined (such as the Voice Visualizer used by Pronovost at Boston University) have shown that even very young children can match the patterns. Pitch indicators have brought about a permanent lowering of pitch in the speech of deaf children at the Tracy Clinic in Los Angeles who sometimes use a very high and unpleasant sounding fundamental.

The discouraging aspect of the work is that none of these devices has significantly improved the intelligibility of the speech of the deaf. The explanation of the failure is fairly clear, but the attainment of success depends on further basic research. The total displays contain too much irrelevant information and omit some necessary information, and the partial displays have not chosen those features which are crucial in determining intelligibility. A basic knowledge of the process of verbal communication, especially the nature of the factors determining intelligibility is lacking, although much research in this areas has been done.

2. The problem of intelligibility

The problem of intelligibility may be dealt with on at least two levels--the phonemic and the semantic. The phonemic level deals with the sounds uttered, and typical testing of phonemic intelligibility is by means of nonsense syllables or words of a prescribed pattern, such as consonant, vowel, consonant, stressed vowel, and consonant. The semantic level deals with the message transmitted, and typical testing would involve whole sentences, or even longer samples of speech. It is of course intelligibility at the semantic level which is the aim of speech training of the deaf. That an utterance can be semantically intelligible, although phonemically greatly distorted is shown by various forms of deviant speech, such as foreign accented speech, ventriloquism, lisping, electronically distorted speech (by filtering, peak clipping, etc.). It is obvious that part, and perhaps even a great part of the semantic intelligibility is carried by the prosodic features of speech--the stress, inflection, and overall timing. There is apparently a certain minimum level of phonemic accuracy combined with correct prosody which will yield semantic intelligibility, and an efficient training program must be based on a knowledge of these minima.

3. Electronic speech processing

Two major areas of relevant research are machine decoding of speech and narrow band speech transmission systems. Machine decoding is relevant to intelligibility at the phonemic level, and could provide a much more satisfactory total visual display than is now available. RCA is developing such a machine, which is quite adequate for adult male voices. Philco is developing a narrow band system, which would yield a much simpler and therefore more easily matchable visual pattern. Both these companies are not of course directing their research toward the problems of the deaf, and do not plan on commercial visual output devices, but the logic of the systems, when completely developed, should lead to valuable training instruments. The models now being developed are adequate to do some analysis of the speech of the deaf.

Significance to Bio-medical Research and the Program of the Institute:

1. Speech of the deaf--at present the congenitally deaf rarely attain intelligible speech, and are thus severely handicapped. Any improvement in their speech would be a great benefit socially, economically, and psychologically.

2. Visual displays might be an aid in speech therapy of hearing subjects.

3. Basic knowledge about the speech recognition or decoding process might result from the studies indicated.

4. Closer contact with the engineering advances in simulation of human sensors may result in the usual benefits of an interdisciplinary approach--an interchange of ideas, methods which may help to solve previously unsoluble problems.

Proposed Course of Project:

1. To complete the study of the accomplishments and failures of visual displays of acoustic information for speech training of the deaf.

2. To suggest ways in which NIH can advance research in this area.

Serial No. NDB(I) 68 OAD 1521
1. Office of the Associate Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: An investigation of the functional response of the retina to light

Previous Serial Number: None

Principal Investigator: Henry G. Wagner, M.D.

Other Investigators: Alan Norton, M.D., Hendrikus Spekreijse, Ph.D., and Myron Wolbarsht, Ph.D.

Cooperating Units: Naval Medical Research Institute, NNMC, Bethesda, Md.

Man Years:

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 1.2 |
| Other: | .8 |

Project Description:

Objectives: To determine the functional mechanisms in the retina which code the physical parameters of light stimuli to the electrophysiological responses in the neurones of the visual pathways.

Methods Employed: Using eyes of suitable experimental animals, intraretina electrical activity will be detected through conventional biophysical technology and correlated with specified optical stimuli.

Major Findings: The receptive field organization of "S" potentials in carp fish retina has been extensively studied. These data disclose that there is full summation of the stimulus over a wide area around the site of response acquisition, possibly over the entire retina. This is true for each chromatically identified component in the three most commonly found types of "S" potentials. These findings are in contrast to the finite limit of summation in the receptive field of retinal ganglion cells.

Using sine wave stimuli to evoke responses in retinal ganglion cells, it has been found possible to differentiate ganglion cells into tonic and phasic types. Phase shift and frequency response characteristics between the stimulus and cell response has been examined in both "S"

potential and ganglion cell responses. Some inferences can be made about the site of nonlinear transformation of the stimulus information to response patterns.

Proposed Course of Project: These studies will be continued. The use of nonlinear stimuli offers unusual opportunities to explore the information processing in the visual system. This concept will be extended to include changes in spatial position of the stimulus as well as chromatic shift to uncover the dynamics of intraretinal actions.

Publication: Norton, A.L., Spekreijse, H., Wolbarsht, M.L., and Wagner, H.G.: Receptive field organization of the S-potential. Science, 160: 3831, p. 1021-1022, May 1968.

Serial No. NDB(1)-68/OAD 1554
1. Office of Associate Director
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Pure and applied mathematics, with special attention to mathematical models of sensory systems

Previous Serial Number: None

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Cooperating Units: Mathematical Research Branch, NIAMD

Man Years:

| | |
|---|---|
| Total: | .25 |
| Professional: | .25 |
| Other: | 0 |

Project Description:

Objectives: To conduct research in pure and applied mathematics, with special attention to mathematical models of sensory systems.

Methods: Mathematical Analysis

Major Findings:

1. The properties of a new type of matrix, called a cuttable matrix, were explored and a number of theorems proved. Cuttable matrices enjoy some simplifying properties analogous to those of reducible matrices.

2. A model of the human visual system proposed by D. H. Kelly was analysed, and some faults found in it. A letter incorporating these results was sent to the Journal of the Optical Society of America.

Proposed course

1. To continue the mathematical research, particularly on the relation of graph and matrix theory.

2. To undertake a study of recent findings in the functioning of the retina, and determine if mathematical methods can clarify the structure and function of this organ.

Publication

Marimont, Rosalind B.: Frequency Doubling. J. Optical Society of America, Vol. 57, No. 7, 968, July 1967.

ANNUAL REPORT
July 1, 1967 through June 30, 1968
Report of the Clinical Director
National Institute of Neurological Diseases and Blindness

Maitland Baldwin, M. D.

During the period of this report, new information has been gained in clinical studies of retinal degeneration, uveitis, and glaucoma. With the Ganzfeld technique and balanced light stimulation, deficits of the rod and cone components in the electroretinogram have been differentiated and correlated with different genetic types of the disease entity. In uveitis the results of antimetabolite therapy in various forms of the disease were reported and the occurrence of serious side effects emphasized. In addition, laboratory studies on patients treated with immunosuppressive drugs, including quantification of immunoglobulin levels, before and after termination of the therapeutic course, the effect of treatment on the primary immune response, estimation of drug induced changes in the induction of delayed hypersensitivity and on the cytologic response established with the skin window technique, provide evidence for several facets of the drug's action. It was thought that the anti-inflammatory properties of these drugs were responsible for encouraging therapeutic results rather than any specific immunosuppression itself.

Acetazolamide has been found an effective prophylactic agent in the prevention of hypokalemic periodic paralysis. Its use has permitted the discontinuation of potassium therapy and yet allowed the victim of this incapacitating disease a prolonged symptom-free period.

A combination of new angiographic and surgical techniques has led to the successful treatment of arteriovenous malformations of the spinal cord which have hitherto been beyond therapeutic capabilities.

In addition, six out of ten patients suffering intractable epilepsy who underwent a combination of cerebral hypothermia and intravenous dilantin are seizure free in periods ranging from one to seven years. Moreover, in a modification of this treatment in which an anticarcinogenic substance, vincristine, is given intravenously along with cerebral hypothermia, two patients with histologically verified cerebral gliomas received therapy and have survived for six years. They are now gainfully employed.

In the past year, the clinical programs have admitted 2,475 patients who have cooperated in various studies. 609 of these were admitted as inpatients and 1,866 within the Outpatient Department. 2,029 consultations were provided to other clinical services within the hospital community and 1,506 diagnostic electroencephalographic examinations were performed. There were 125 major operative procedures on the nervous system and 250 muscle biopsies, while 103 patients underwent extensive physiological monitoring related to their neurological disease entities.

The clinical staff of the Institute gratefully acknowledges the cooperation and assistance of all Departments of the Clinical Center, without whose collaboration their work would be impossible.

Annual Report
July 1, 1967 through June 30, 1968
Medical Neurology Branch, IR
National Institute of Neurological Diseases and Blindness

W. King Engel, M.D.
Chief, Medical Neurology Branch

## Clinical Investigation Program

Introduction: An inter-related multidimensional attack on the chosen target diseases is emphasized in our application of basic research techniques to clinical neurologic problems. The techniques consist of: histochemistry, biochemistry, immunology, autoradiography, electron microscopy, tissue culture, and electromyography. In the human neurologic disorders studied, these techniques support thrusts to seek: (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, sub-forms; (c) specific or symptomatic therapeutic response; and (d) animal models of the human pathophysiologic states.

For the clinical investigations, 246 patients were admitted for a total of 6,735 patient days, and there were 773 out-patient visits. There were 250 muscle and brain biopsies obtained. The neurologists provided 417 consultations to other departments, performing the indicated myelograms, pneumoencephalograms, and cerebral angiograms on those patients.

The one-year approved residency training program in neurology has continued; medical students and residents from Howard University were taught clinical neurology weekly; and investigators and technicians came as guest workers to learn clinical research techniques in neurology, especially the application of enzyme histochemistry to human neuromuscular disease. The collaborative research program in neuromuscular disease with the Department of Neurology, Warsaw Medical Academy has continued under the PL-480 program.

Central Nervous System Degenerations: Contrary to the literature, a case of well documented subacute sclerosing panencephalitis (with elevated measles antibody titers) was shown to undergo marked spontaneous improvement without antiviral or immunosuppressive therapy, antibody titers remaining elevated after improvement (with PRB). In other cases of this disorder, normal cellular defense mechanisms were demonstrated. A new familial CNS and peripheral nervous system disease associated with acanthocytosis but normal lipoproteins has been demonstrated (with NHI and NIAMD). In ataxia teleangectasia the impaired lymphocyte transformation has been found susceptible to a factor from normal serum.

Motor Neuron Diseases: In infantile spinal muscular atrophy (ISMA) two sporadic cases showed eosinophilic nuclear inclusion bodies, raising the question of a viral factor, but as yet this disease has not been transmissible to animals. Histochemical studies reveal lack of a disease-specific change in the motor neurons of the brain stem and spinal cord in ISMA. In amyotrophic lateral sclerosis (ALS) it was demonstrated that there is no abnormality of the following metabolic processes: synthesis and catabolism of IgA and IgG, catabolism of caeruloplasmin and catabolism of albumin, each with isotope-labeled protein (with NCI); plasma lipoprotein content, by paper electrophoresis; plasma fatty acids, and specifically phytanic acid, by gas-liquid chromatography (with NHI); serum arginine content; aminoacid excretion, by aminoacid analysis of urine. The intolerance of oral glucose in about 30% of ALS patients was found not disease specific, being present in about the same percent of patients with other neuromuscular diseases. Studies of tolbutamide tolerance, insulin tolerance, and assay of plasma insulin (with U. Rochester) indicate (a) no consistent abnormality of pancreatic endocrine function in ALS, and (b) loss of muscle bulk is the best explanation of glucose intolerance in the various neuromuscular diseases. Atypical courses of seemingly typical forms of ALS have been demonstrated, one with nearly complete recovery. Double-blind, controlled clinical trials using quantitative muscle testing showed lack of therapeutic benefit from: fresh human plasma; vitamin E plus pancreatic extract; lipoic acid; prednisone; and adamantine (an anti-viral agent). Histochemical studies of CNS tissue show lack of a specific histochemical change in the motor neurons of ALS. Histochemical studies of this and other human material demonstrated abundant phosphorylase in motor neurons and subpial glia, both presumably normal and probably indicative of high glycolytic activities of those cells.

Peripheral Neuropathy: A new metabolic cause of familial recurrent neuropathy, viz. α-lipoprotein deficiency (Tangier disease), has been reported (with NHI). In Refsum's disease (hyperphytanicacidema) we have provided the detailed clinical evaluation of patients undergoing therapeutic trial of a low phytol diet by NHI. Electron microscopy reveals the principal defect in peripheral nerve of Refsum's disease is in Schwann cells, which show (a) hypertrophy and (b) morphologically distinctive "crystalline" inclusions. In primary familial amyloidosis, which is usually associated with a sensory neuropathy, the vitreous veils were shown histochemically to be composed of amyloid and originate from retinal arteries (with Ophth.).

A new histochemical method of demonstrating the delicate cytoplasmic processes of Schwann cells in single teased nerve fibers has been introduced and applied to events following crush

injury of nerve, leading to the hypothesis of a recoiling phenomenon of axons. Target fibers were described electron-microscopically, and the ultrastructural details correlate well with the histochemical results.

Animal studies demonstrated the validity of myosin ATPase as the best histochemical signature of muscle fiber type following experimental denervation, in agreement with the conclusions from study of human disease material. In animals, the ability of nerve to influence muscle was demonstrated in the following ways: (a) conversion of normal newborn guinea pig soleus muscle from mixed to uniform histochemical pattern at 6 weeks - with prevention of that change by neonatal denervation or cordotomy; (b) reversion of adult guinea pig soleus from uniform to mixed histochemical pattern in 6 months following denervation and in only one month following corodotomy; (c) ability of "foreign" nerve fibers to convert muscle fiber types in the guinea pig soleus; and (d) correlation of such histochemically partially converted soleus muscles with changes of physiologic function. "Type grouping" of muscle fibers, demonstrated empirically to be a sign of chronic denervation in humans, was produced experimentally in animals by nerve section and subtotal reinnervation to confirm the hypothesis of pathogenesis proposed from the human material.

With autoradiography, axonal flow rates have been calculated in cat motor and proximal and distal sensory nerves for normals and two types of toxic axonal neuropathy.

Neuromuscular Junction - Myasthenia Gravis: Marked therapeutic success achieved with long-term ACTH in a case of atypical childhood myasthenia gravis (in adults, ACTH is used short-term). Immunological characterization and demonstration of distinction between anti-nuclear factor and anti-muscle factor in sera of myasthenia gravis patients was achieved using highly specific anti-sera made in this Branch. The former could be present in any immunoglobulin fraction and the latter only in IgG.

Congenital "Myopathies": Two more new disorders have been reported: (a) segmental loss of cross-striations, in which there are focal areas of myofibrillar disorganization and absence of mitochondria and aqueous sarcoplasmic enzymes; (b) type I muscle fiber hypotrophy with central nuclei, the first case being fatal at 18 months of age, and the second being in a non-progressive condition affecting an 11 year old boy. An experimental model of selective hypotrophy of one fiber type with central nuclei was produced by neonatal sciatic denervation of the rat gastrocnemius, raising the possibility that the human disorder is not a myopathy as it might seem on first impression but rather due to an alteration of the neural influence of the muscle fibers. In rod (nemaline) myopathy a new progressive fatal form of mid-childhood

has been found. It has been demonstrated that rod myopathy is a quantitative, not qualitative, diagnosis. An experimental model with electron-microscopically typical rods has been produced by tenotomy of cat muscle, thus providing a source for future analysis of the composition and pathogenesis of the rods.

Other Myopathies, including Dystrophies: A new classification of biochemical abnormalities in myopathies, based on (a) disease specificity and (b) importance of pathogenesis, has been presented. In muscle phosphorylase deficiency (McArdle's disease) it has been demonstrated that a heterozygous (carrier) state is not biochemically detectable in muscle. The hypercatabolism of IgG, previously shown to be a specific feature of myotonic dystrophy, has now been shown to be due to hypercatabolism of the H chain of the IgG molecule (with NCI). "Needle myopathy" has been shown to be a significant clinico-pathologic, iatrogenic phenomenon. In Duchenne dystrophy the basic histochemical expression of the carrier state is atrophy and architectural derangement of scattered or sometimes slightly grouped muscle fibers, containing high RNA and oxidative enzyme staining, and affecting both histochemical fiber types.

Analysis of human biopsies by histogram technique to study fiber diameters has led to the definition of normal diameters by fiber type for each sex and the abnormalities of diameter of each fiber type in the various sorts of neuromuscular diseases.

Inflammatory Myopathy - Polymyositis: An excellent clinical response (one patient became clinically normal) has been achieved with a newly applied therapeutic agent for this disease - azathiaprine - an antimetabolite and immunosuppressive agent.

Periodic Paralysis: What appears to be the best prophylactic agent thus far available in hypokalemic periodic paralysis has been found in the form of acetazolamide. It has allowed patients to discontinue potassium therapy and still remain virtually attack free. It benefits most but not all of such patients studied. Balance studies have failed to provide a satisfactory explanation of its mechanism of action in this disease. It has become clear that vacuoles in periodic paralysis are not an integral aspect of the pathogenesis but rather an epiphenomenon, because they can be abundant in strong muscles and absent in very weak muscles. Hyperaldosteronism was demonstrated in a patient with thyrotoxic periodic paralysis, with reversal of both abnormalities by antithyroid treatment but of neither with reserpine treatment alone.

Methodology: The electron probe has been used to detect invisible end-products of histochemical reactions, apparently for the first time, thus opening the field of histochemistry to the use of any end-product, visible or invisible by ordinary light

microscopy, but adequate for the electron probe so long as it contains an element with an atomic number of boron (5) or greater. When such an element is deposited in the tissue as a result of an enzymatic reaction, that reaction can act as a multiplier producing sufficient amounts to be easily detected by the electron probe. With the probe, enzyme differences between the two human muscle fiber types were roughly quantitated. The ability to tease out single human glycerated muscle fibers and to type them histochemically for correlation with physiologic parameters has been achieved. There are no demonstrable physiologic differences in rate of tension development, maximal shortening velocity, or maximum tension output between the two histochemical fiber types in such human single fiber preparations. The tonic fiber system of amphibian muscle has been identified histochemically.

## Neuroradiology

Radiographic diagnosis: Selective arteriography of the spinal cord vessels is now established as the diagnostic procedure of choice in cases of arteriovenous malformations of the spinal cord. Angiographic demonstration of the arteries feeding these malformations has been possible in 15 cases. This technique has been extended to other pathological conditions. Thus, displacement of the arteria radiculo-medullaris magna (Adamkiewicz) and the anterior spinal artery has been observed in five patients with space-occupying lesions (tumors and others) in and around the spinal cord. This displacement made possible in one case the identification of the upper limit of a cord tumor, which had not been recognized by means of other radiodiagnostic studies (myelography). Arteriography of the spinal cord has also allowed the visualization "in vivo" of collateral flow through dilated spinal cord arteries ("the hypertrophied spinal artery syndrome") in association with aortic coarctation and extraspinal arteriovenous shunts. The recognition of these dilated spinal cord arteries is important because they may be responsible for cord compression, and if ruptured, for spinal subarachnoid bleedings. Finally, the possibility of consistently demonstrating the artery of Adamkiewicz (main blood supplier from aorta to thoraco-lumbar cord) by the technique of selective arteriography furnishes the surgeon undertaking any procedure involving the descending thoracic and upper abdominal aorta with valuable information concerning the cord vascularization. This information may be helpful to prevent the dreaded spinal cord damage after aortic surgery.

Expansion of the intrasellar subarachnoid extension (cisterna intrasellaris) is responsible for the anatomo-radiographic syndrome of so-called "empty sella" and its related clinical conditions (hypo- and dyspituitarism, spontaneous cerebrospinal fluid rhinorrhea). Additional sella turcica measurements of

hypopituitaric dwarfs who are being treated with growth hormone have been collected and are being evaluated to establish the effect, if any, of the growth hormone on their small sella.

Isotopic diagnosis: For brain scanning, $^{99m}$Tc Technetium human serum albumin ($^{99m}$Tc-HSA) is now a routinely used tracer. Lesions missed with other tracers have been demonstrated by $^{99m}$Tc-HSA scanning, and in some instances, vice-versa. Thus, brain scanning with multiple isotopes improves the reliability of this diagnostic procedure.

The NIH Tetrascanner and the gamma camera (Anger type) have been compared for over a year. It has been confirmed that these two devices are complementary to each other. Rapid studies require the camera, but if detail is needed the Tetrascanner resolution is indispensable.

Further experience has been gathered with the now well established techniques of isotope-cisternography and isotope-ventriculography. RIHSA or high specific activity $^{99m}$Tc-HSA are both being used as tracers, either separately or occasionally combined. $^{99m}$Tc-HSA has been confirmed as the tracer of choice for the study of the cerebrospinal fluid rhino- and otorrheas, but RIHSA is still the preferred radiopharmaceutical for the cisternographic study of the communicating (particularly the so-called "normotensive") hydrocephalus.

A theoretical evaluation of the possible approaches to scanning tomography (a method to obtain radioisotope scans of uniplanar sections of a region of the body) has been undertaken, in cooperation with the Case Institute of Technology. A mathematical analysis of the three fundamental parameters - sensitivity, resolution and efficiency - of the various devices, already available or proposed, is well under way with the help of a computer. When this analysis is completed, we will be able to appraise the relative merits and pitfalls of the various methods, with a view toward building a prototype Tomoscanner.

## Applied Pharmacology

Excitation-contraction coupling: The ionic mechanisms for the sequence of physiological events that lead to contraction of slow skeletal muscle have been further delineated. Evidence has been obtained that $Na^+$ current is one of the sequential steps between excitation and tension development. The $Na^+$ currents are under control of $Ca^{++}$ ions at a surface phase level and affect subsequent processes at a deeper phase, probably by $Ca^{++}$ release. The data do not indicate that other monovalently charged ions cannot function in place of $Na^+$ when the muscle system environment is non-physiological. Potassium and chloride are not functional at the same

phase level as $Na^+$. Calcium ions function at not less than 3 phase levels and possibly at as many as seven. The $Na^+$ current under $Ca^{++}$ control is not analogous to the $Na^+$ current of fast muscle or nerve action potentials because neither tetrodotoxin nor procaine blocks the $Na^+$ current of slow muscle. d-Tubocurarine and atropine also fail to affect the slow muscle $Na^+$ current effect and it therefore is not similar to end plate Na current which follows neurohumor transmitter release. The collection of these data was made possible by investigating the substitution of $Na^+$ by quaternary nitrogen compounds and buffer amines capable of protonation at physiological pH's. The studies showed that muscle membranes which were adequately blocked against the effect of quaternary nitrogen compounds can be reactivated to respond by the removal of $Ca^{++}$. Certain buffer amines have been found which adequately substitute for $Na^+$ as to monovalent charge but fail as substitutes for the physiological action of $Na^+$. Some nitrogenous sulfonic acids were found that can adequately substitute for chloride.

Venoms: Extension of the PL-480 collaborative program with Ein Shams University, Cairo, Egypt provides for continuation of the obtaining and housing of poisonous animal species of Egypt and collection of their venom, as well as extending the geographical area of species collection to North and West Africa. The program involves the development of improved and polyvalent antivenom and the ancillary laboratory research at the site of venom collection. The neurotoxic and neuropharmacological activities of the venoms and antivenom are investigated in this Branch and elsewhere at NIH. It has been found that venoms from each species of the genus Naja are capable of damaging the mammalian blood brain barrier and changing its permeability to large dye molecules. This neurotoxicity is of secondary importance to the initial pharmacological actions of the Naja venoms which cause neuromuscular blockade and cardiovascular insufficiency. The latent direct effect on muscle and decrease in electrical activity of the brain also appear related to cardiovascular deterioration. Studies of the acute toxicities of a large variety of venoms have clarified some of the problems of standardization as to source, storage and method of preparation. An example of physiological subspeciation has been demonstrated in Naja haje (Egyptian cobra).

Muscle contraction: The adaptive and trophic mechanisms related to mechanical output of mammalian twitch muscle have been investigated using two chronic rat preparations. Changes in the mechanical output of muscle immobilized by joint fixation were primarily confined to the fast muscle, which showed decrease in contractile speed and capacity for tension development. The only change observed in the immobilized slow muscle was in the tetanus:twitch tension ratio, interpreted as muscle speeding. Implanting a "fast nerve" to slow muscle increased the contractile rate and produced hypertrophy, but did not increase the tension-producing capacity.

Improved instrumentation has permitted reinvestigation of the series elastic component in both slow and fast twitch muscle. Mathematical and statistical treatment of the data have provided a generalized expression for their elastic properties. Suspected discrepancies in the energy output of the elastic elements have been confirmed, necessitating revision of the functional model of skeletal muscle.

Force-velocity measurements, which relate directly to the active contractile components of the muscle, have been obtained for several muscles in two species. Preliminary analysis of data has shown a correlation between muscle speed and force-velocity parameters, which indicates that derivation of such a classification index is possible.

Serial No. NDB(I)-62 MN/OC 915(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Histochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Michael H. Brooke, M.D.
Jerome S. Resnick, M.D.

Other Investigators: Eugene Martin
Richard L. Irwin, Ph.D.
David S. Dahl, M.D.

Cooperating Units: Philips Electronic Instruments
Mount Vernon, New York

Man Years:

| | |
|---|---|
| Total: | 3.2 |
| Professional: | 3.2 |
| Other: | 0.0 |

Project Description:

Objectives: (1) To study the cellular and subcellular localization of a variety of histochemical reactions in normal human skeletal muscle and neurons and to see how they are altered in neurologic diseases. (2) To develop new histochemical techniques and to analyse mechanisms of the reactions. (3) To correlate histochemically defined types of muscle fibers with physiologic, ultrastructural, and biochemical properties. (4) To derive a method for quantitating the findings in a muscle biopsy so that the abnormality may be expressed simply and numerically in the same way that the results of electromyography may be expressed numerically. (5) To apply the above results to give information which may be used diagnostically. (6) To use the electron probe for expanding the boundaries of enzyme histochemistry.

Methods Employed: Histochemical techniques for a number of reactions are being done on muscle and nerve tissue - they have been outlined in previous reports.

Histograms were constructed of the transverse diameter of muscle fibers in a biopsy. At least 200 fibers in each muscle biopsy specimen of more than 1000 patients were analysed. From the histogram the following figures were derived for each fiber type: (1) average diameter and standard deviation, (2) a set of numbers, derived mathematically and representing the proportion of fibers which were abnormally small and those which were abnormally large. The ratio of type I fibers to type II fibers was also calculated.

In frog rectus abdominus (containing both tonic and twitch muscle fibers), histochemistry revealed dorsal and ventral layers of different fiber types, only one layer was selectively cut in different specimens and physiologic properties of the intact fibers analysed and correlated with the histochemical results. The same procedure was followed to identify the tonic fiber bundles in ileofibularis, both heads of semitendinosus, and gastrocnemius.

From human muscle biopsies, after glyceration, individual fibers are teased. From the same single fiber, a portion is typed histochemically, another part studied physiologically, and a third portion fixed for electron-microscopic examination. Correlations of the 3 approaches are made.

Human skeletal muscle tissue was incubated using the histochemical technique developed in this Branch for EDTA-activated ATPase of muscle fibers but only to the point of invisible $CaHPO_4$ precipitate. These sections were then analysed with the electron probe microanalyser (Norelco Model AMR/3, Philips Electronic Instruments) and compared with serial sections of the same tissue incubated in additional cobalt and sulfide solutions to obtain a colored end-product. Electron-beam scanning displays were made for characteristic X-rays of elements in the tissue; sample current and back-scattered electrons were also analysed. Averaged countings over a 1-μ spot were taken for the two histochemical fiber types. Native (unreacted) sections were also analysed with the electron probe for Ca, P, Fe, K, and Na.

Patient Material: Medical Neurology Branch patients as well as consultation patients and patient biopsy material submitted by outside physicians.

Major Findings: Histochemistry of muscle biopsies continues to provide important information in neuromuscular diseases. The use of histograms as part of the total evaluation of muscle biopsies in various diseases revealed that the two histochemical fiber types behave differently under various conditions, reactions not easily apparent without histograms.

The following were considered to be the items of "new" information from the histogram analyses: (1) Type II fibers are smaller in normal females than in males. They appear to increase in size in association with work and to decrease in size following inactivity. This inactivity may be due to functional or organic causes ranging from bed-rest to pyramidal disease. The type I fibers are not thus affected. (2) The characteristic finding of chronic familial peripheral neuropathy in adults and denervating diseases in children is the presence of large type I fibers. This change differentiates these conditions from simple peripheral neuropathies in adults and from ALS. (3) Over 90% of patients with myotonic dystrophy have preferential atrophy of the type I fibers of the biceps and vastus lateralis. None has these changes in the gastrocnemius. (4) Duchenne dystrophy is characterized by type I fiber predominance. (5) In floppy babies the biopsy is useful in differentiating those with muscular difficulty associated with central nervous system damage and a "poor" prognosis from those without CNS damage and a better prognosis. In addition to the above, the classical concepts of muscle pathology (such as the variability of fiber size in the myopathies) were given numerical confirmation.

The individual tonic muscle fibers of the frog were identified histochemically for the first time, using a battery of histochemical techniques combined with physiological studies after selectively cutting muscle fibers.

A technique has been developed for studying the physiologic, histochemical, and electron-microscopic properties in the same individual, teased, human glycerated muscle fiber. It has been demonstrated that histochemical typing of such fibers is possible.

It was clearly demonstrated that the electron probe can be used to detect otherwise invisible histochemical end-products. Rough quantitation with the probe indicated that type I muscle fibers have about 100 times the amount of end-product deposited by the EDTA-activated ATPase reaction as type II fibers. Native sections have only small amounts of Ca, P, K, Fe, and Na, and no detectable differences between the two fiber types.

Significance to Bio-Medical Research and the Program of the Institute: It is hoped that the standardization and quantitation of muscle biopsy findings will allow the more accurate interpretation of biopsies from patients with undetermined diseases.

Of particular interest are the findings of an apparently selective involvement of one histochemical type of muscle fiber, suggesting that the particular characteristics of metabolism of that type of muscle fiber are more susceptible to a given disease

process. This permits us to ask why they are more susceptible and conversely, what makes the others more resistant? Detailed analysis of single teased human muscle fibers will provide more information on this question.

This study appears to represent the first application of the electron probe to detect invisible end-products. Ability to detect elemental precipitates with the electron probe (applicable to all elements with the atomic number of boron, 5, or above) can reduce the number of steps in many histochemical reactions, as in the one described, thereby lessening the danger of false localization. Of perhaps greater importance is that development of new histochemical reactions with otherwise invisible elements in the reaction product is now possible.

Proposed Course of Project: The work is to be continued by investigating the way in which animal experimentation can reproduce some of the above findings. The detailed results of histochemical and cytochemical evaluation of muscle biopsies from about 1500 patients, averaging 10 different histochemical reactions each, are being compiled into a book Muscle Histochemistry, due to be completed soon. Further correlations of the results from light-microscopy with those of electron-microscopy, single fiber physiology, and biochemistry will be made in human muscle diseases. Additional reactions will be studied with the electron probe.

Honors and Awards: None

Publications: Engel, W.K., and Irwin, R.L.: A histochemical-physiological correlation of frog skeletal muscle fibers. Amer. J. Physiol. 213: 511-518, Aug. 1967.

Engel, W.K., Resnick, J.S., and Martin, E.: The electron probe in enzyme histochemistry. J. Histochem. Cytochem. In Press.

Serial No. NDB(I)-62 MN/OC 917(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Martin A. Hatcher, M.D.
Robert C. Griggs, M.D.
Paul W. Hathaway, M.D.

Other Investigators: Harold M. Fullmer, M.D.
Gerald S. Lazarus, M.D.

Cooperating Units: Laboratory of Histology and Pathology, NIDR

Man Years:

| | |
|---|---|
| Total: | 2.2 |
| Professional: | 1.8 |
| Other: | 0.4 |

Project Description:

Objectives: To seek and analyse biochemical abnormalities of neuromuscular and other neurologic diseases.

Methods Employed: Blood and muscle biopsy extracts of patients were studied with a variety of techniques:

a. Lactate, pyruvate, creatine phosphokinase (CPK) of blood.

b. Enzymes of glycogen metabolism in muscle - phosphorylase, glycogen, amylo 1,6 glucosidase, phosphoglucomutase, and acid and neutral maltase.

c. Metabolism of muscle studied by incubating various $C^{14}$ labeled substrates - glycolytic and Kreb cycle intermediates, fatty acids, and amino acids - with human muscle homogenates and measuring the amounts of labeled $CO_2$ produced.

d. The arylsulfatase assay of urine was set up to study typical and atypical cases of metachromatic leucodystrophy.

e. Creatine phosphokinase technique in acrylamide gel to analyse isozymes in the serum.

f. Collagenase in nervous, muscular, and other tissues by several methods.

Patient Material: Patients with neurologic diseases.

Major Findings:

a. Several patients with McArdle's disease were diagnosed. In one family with 3 affected children (complete absence of muscle phosphorylase), the parents were proved to have normal phosphorylase thus demonstrating lack of a heterozygous (carrier) state determinable by muscle enzyme study. Various infusion studies on the patients with McArdle's disease were performed to document with lactate determinations the successful by-pass of the metabolic block. From this study it was suggested that fructose was preferable to glucose as a therapeutic agent. Many patients suspected of having McArdle's disease or other glycolytic defects were screened and found to be normal.

b. Further studies were performed on a patient suspected of having a biochemical defect in lactate metabolism associated with abnormal mitochondria by electron-microscopy. A variety of physiological studies were performed involving exercise with concomitant measurement of lactates, pyruvates, glucose, and respiratory quotients. He appears to have exercise-induced hyperlacticacidemia; whether it is overproduction or under utilization is being determined. An assay was set up to determine the "normal" reaction of human muscle homogenate when incubated with various $C^{14}$ labeled substrates - glycolytic and Krebs cycle intermediates, fatty acids, and amino acids. When this test is standardized, it will be applied specifically to the muscle of the above-mentioned patient in order to further define his metabolic abnormality. It will also be applied to a variety of other biopsies. This test has many possible applications to the study of muscle metabolism in general as well as in specific muscle diseases.

c. A new classification of biochemical abnormalities in neuromuscular disorders was presented, based on (a) disease specificity and (b) importance to pathogenesis.

d. Variations in the patterns of serum creatine phosphokinase isozymes in normals and in patients with various neuromuscular disorders were described.

Significance to Bio-Medical Research and the Program of the Institute: Elucidation of biochemical abnormalities, particularly in the realm of enzymes and other proteins, is vital to the understanding of neurologic and neuromuscular diseases, and in seeking means of therapy. Putting the various abnormalities in proper perspective regarding pathogenesis and disease specificity, as with the classification proposed, is often neglected but is vital to planning future investigative and therapeutic procedures.

Proposed Course of Project: The above techniques will continue to be perfected and applied to muscle from a variety of human neuromuscular disorders to seek further details of biochemical defects therein.

Honors and Awards: Principal investigator was invited chairman of opening symposium on neuromuscular diseases, 2nd International Congress of Neuro-Genetics and Neuro-Ophthalmology, Montreal, Sept. 1967.

Publications:

Engel, W.K., and Hatcher, M.A.: Evaluating significance of biochemical abnormalities in inherited neuromuscular disorders. Proceedings of the 2nd International Congress of Neuro-Genetics and Neuro-Ophthalmology. In Press.

Fullmer, H.M., and Lazarus, G.S.: Collagenase in human and rat bone: a preliminary report. Israel J. Med. Sci. 3: 758-761, 1967.

Lazarus, G.S., Brown, R.S., Daniels, J.R., and Fullmer, H.M.: Human granulocyte collagenase. Science 159: 1483-1485, 1968.

Lazarus, G.S., Fullmer, H.M., Oliver, C.H., Multz, C.V., Barth, W.F., Decker, J.L., and Kamin, E.J.: Collagenase activity of human synovium. Proceedings of the IV Inter-American Congress of Rheumatology. In Press.

Wolintz, A.H., Engel, W.K., and Summers, T.F.: Isoenzymes of creatine phosphokinase determined by acrylamide gel electrophoresis. J. Mt. Sinai Hosp. 35: 174-178, Mar-Apr. 1968.

Serial No. NDB(I)-63 MN/OC 1034(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Myopathies

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators: Jerome S. Resnick, M.D.
Michael H. Brooke, M.D.

Cooperating Units: None

Man Years:

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|---|---|
| Total: | 2.7 |
| Professional: | 2.7 |
| Other: | 0.0 |

Project Description:

Objectives: To more fully elaborate the clinical, histochemical, biochemical, and ultrastructural abnormalities of patients with the various myopathies, To further sub-classify patients in each category using those techniques. To treat these disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

Methods Employed: Most of the details of techniques applied to human material are described in the various methodological projects. The effects of needles inserted into guinea pig muscle, just as is done in a human EMG study or therapeutic injection, were analysed histochemically. Neonatal rats had sciatic neurectomy on one side and the gastrocnemius and soleus muscles of both sides were studied histochemically for up to 8 weeks thereafter.

Patient Material: Medical Neurology Branch patients.

Major Findings: Two new types of neuromuscular disease have been found. One consists of focal loss of muscle fiber cross striations in a 14 year old girl with a very mildly progressive

congenital weakness. In the focal lesions, myofibrils are disorganized with loss of neat alignment of filaments, and mitochondria and aqueous sarcoplasm (e.g. glycogen and phosphorylase) are absent. The possibility of a basic mitochondrial defect is being further explored by electron-microscopy.

The other disease, in a child with congenital weakness fatal at age 18 months, consists of complete hypotrophy of type I muscle fibers, with central nuclei, associated with normal motor neurons of the spinal cord and brain stem. The former disease is probably an unusual type of myopathy. It is uncertain whether the latter is a muscle disease or is secondary to abnormal motor innervation of the muscle fibers because a similar selective hypotrophy of one muscle fiber type, with central nuclei, was produced by neonatal denervation of rat gastrocnemius as an animal model. The second possibility is actually favored. A second, milder case of type I hypotrophy with central nuclei was found in an eleven year old boy with essentially non-progressive weakness.

In rod (nemaline) myopathy, benign congenital and fatal late-onset forms were both originally described from this Branch; now a new form fatal in mid-childhood has been found. Additional studies indicate that the rods themselves are not unique to these patients, as a few scattered rods have been found in occasional patients with various other diseases. It remains however that abundant rods with the characteristic electron microscopic Z-band location and longitudinal periodicity, in the absence of other major pathology are diagnostic of rod myopathy, which is now a quantitative, not qualitative diagnosis.

The clinical importance of needles (electromyographic or hypodermic) inserted into a muscle hours to weeks before the biopsy in their ability to produce a myopathic picture, with or without inflammation, was demonstrated experimentally and in patients. The introgenic artifacts likely to be engendered by the practice of biopsying a recently EMGed muscle were reported and termed "needle myopathy."

In carriers (heterozygotes) of Duchenne muscular dystrophy, subtle histochemical abnormalities have been found, which differ from the more gross (and possibly factitious) ones recorded by other observers. The basic changes are fibers, scattered or in very small groups, of both histochemical fiber types with the myofibrillar ATPase reaction, showing slight atrophy, nuclear activation, and increased staining for RNA and mitochondrial oxidative enzymes.

The excellent results of treating inflammatory myopathies (polymyositis) with the immunosuppressive agent azathiaprine has been detailed in another project (immunology).

In myotonic dystrophy other investigators have reported very high insulin responses to administered glucose. With NIAMD, we have confirmed this response, and extended the study to define insulin and glucose responses to tolbutamide, arginine, and exogenous insulin in myotonic dystrophy.

A new family with central core disease has been reported. Histochemical and electron-microscopic studies show (a) cores confined to type I muscle fibers, and (b) cores qualitatively like target fibers (see EM project for target fiber details), differing only quantitatively, enhancing our previous hypothesis that central core disease may not be a myopathy but rather due to defective neural influence.

Significance to Bio-Medical Research and the Program of the Institute: The myopathies represent a group of disorders rather similar clinically, the detailed differences of which have only begun to be worked out in the recent few years. One of the major methods for distinguishing these disorders is by histochemistry of the muscle biopsy. Other methods include electron microscopy, biochemistry, and immunology. Hence, the distinguishing of various new forms of disease represent a step toward the overall understanding of this group of disorders. Identification of a possible neurogenic component to "myopathies" shifts 180° the avenues of investigation of pathogenesis. It is only when the detailed differences of the various forms of myopathy are analysed that the pathogenesis, etiology, and treatment can be understood. Identification of characteristic histochemical and biochemical defects in the myopathies provides leads to further explore the pathogenesis and possible treatment.

Proposed Course of Project: The studies underway are part of a long-term project which will continue for several years.

Honors and Awards: Two of the following papers written were by invitation. Principal investigator was invited co-chairman and organizer of the scientific program of International Symposium "Exploratory Concepts in Muscular Dystrophy and Related Disorders".

Publications:

Engel, W.K.: A critique of congenital myopathies and other disorders. In Milhorat, A.T. (Ed.): Exploratory Concepts in Muscular Dystrophy and Related Disorders. New York, Excerpta Medica Foundation, 1967, pp. 27-40.

Engel, W.K.: Focal myopathic changes produced by electromyographic and hypodermic needles ("Needle Myopathy"). Arch. Neurol. 16: 509-511, May 1967.

Engel, W.K.: Muscle biopsies in neuromuscular diseases. Pediat. Clin. N. Amer. 14: 963-995, Nov. 1967.

Engel, W.K., Gold, G.N., and Karpati, G.: Type I fiber hypotrophy and central nuclei - A rare congenital muscle abnormality with a possible experimental model. Arch. Neurol. 18: 435-444, Apr. 1968.

Engel, W.K., and Karpati, G.: Impaired skeletal muscle maturation following neonatal neurectomy. Develop. Biol. In Press.

Serial No. NDB(I)-63 MN/OC 1037(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Microbiology Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

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| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: To study the growth characteristics, histochemical reactivities, immunologic properties, and electron-microscopic details of skeletal muscle and motor neurons grown in tissue culture. The muscle is either chick embryo or normal or abnormal biopsied human muscle.

Methods Employed: Standard tissue culture techniques are used as a basis, modified as necessary to obtain good growth from human skeletal muscle and neurons. Applied to the cultured cells are other techniques active in the Branch, e.g. histochemistry, electronmicroscopy, and immunology.

Patient Material: A portion of the muscle biopsy from certain patients with neuromuscular disease is taken for culture purposes.

Major Findings: This project was inactive this past year but will be reactivated in the coming year when more space becomes available.

Significance to Bio-Medical Research and the Program of the Institute: In addition to determining the histochemical

properties of normal muscle and its growth characteristics in vitro, the studies of abnormal muscle will be used to seek disturbed growth patterns and, more particularly, to determine ways of improving deficient growth. Agents found to improve deficient growth in vitro will then be tested for their usefulness in the patient to promote growth of abnormal muscle or to retard its degeneration. As a foundation for understanding phenomena in human muscle, chick embryo material has been studied. Study of motor neurons grown in vitro is part of a multilateral approach to diseases which preferentially involve human motor neurons, such as amyotrophic lateral sclerosis.

Proposed Course of Project: Further studies of normal and abnormal human muscle in vitro will be done. Definitive studies, starting with histochemistry, will be applied to the cultured motor neurons.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-63 MN/OC 1039(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators: Jon D. Dorman, M.D.
Robert I. Levy, M.D.
Frederic Q. Vroom, M.D.
Daniel Steinberg, M.D.
Thomas A. Waldmann, M.D.
Donald S. Schalch, M.D.

Cooperating Units: Laboratory of Metabolism, NHI
Medicine Branch, NCI
University of Rochester School of Medicine

Man Years:

| | |
|---|---|
| Total: | 3.6 |
| Professional: | 3.5 |
| Other: | 0.1 |

Project Description:

Objectives: In ALS and other diseases affecting the lower motor neurons, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, sub-forms; (c) specific or symptomatic therapeutic response; and (d) animal models of the human pathophysiologic states.

Methods Employed: Generally, the techniques of histochemistry, biochemistry, autoradiography, electron-microscopy, immunology and electromyography, as detailed in other project reports. Specific examples, as follows. Double-blind therapeutic trials, the efficacy of which is judged by clinical testing, functional evaluation, and serial quantitative evaluation of muscle function using an apparatus designed by us. Carbohydrate metabolism studies - glucose, tolbutamide, and insulin tolerance tests; assay of plasma insulin (with U. Rochester). Lipoprotein studies (with

NHI). Analysis of motor areas of brain and spinal cord by histochemistry and electron-microscopy, with methods described in other project reports. Biochemical assays of serum fatty acids (with NHI) and turnover of serum protein fractions (with NCI). Search for virus by electron-microscopy, and by inoculation of tissue cultures and animals (with PRB and C&FR).

Patient Material: Medical Neurology Branch patients.

Major Findings:

MOTOR NEURON DISORDERS

The intolerance of oral glucose in about 30% of ALS patients was found not disease specific, being present in about the same percent of patients with other neuromuscular diseases. Studies of tolbutamide tolerance, insulin tolerance, and assay of plasma insulin (with U. Rochester) indicate (a) no consistent abnormality of pancreatic endocrine function in ALS, and (b) loss of muscle bulk is the best explanation of glucose intolerance in the various neuromuscular diseases.

In amyotrophic lateral sclerosis (ALS) it was demonstrated that there is no abnormality of the following metabolic processes: synthesis and catabolism of IgA and IgG, catabolism of caeruloplasmin and catabolism of albumin, each with isotope-labeled protein (with NCI); plasma lipoprotein content, by paper electrophoresis; plasma fatty acids, and specifically phytanic acid, by gas-liquid chromatography (with NHI); serum arginine content; aminoacid excretion, by aminoacid analysis of urine.

The apparatus we have designed and built for quantitating muscle strength has been shown to give reproducible results in normal controls and patients. It serves as one aspect of evaluating ALS patients who are participating in our therapeutic trials.

In ALS, double-blind therapeutic trials showed that vitamin E plus pancreatic extract, lipoic acid, adamantine (an antiviral agent), prednisone, guanidine, and intravenous plasma were not of therapeutic value, as judged by clinical evaluation and our quantitative muscle testing machine.

In infantile spinal muscular atrophy (ISMA) two sporadic cases showed eosinophilic nuclear inclusion bodies, raising the question of a viral factor. Attempts to transmit an infectious agent to tissue cultures and mammals are underway, using material from patients with ALS, infantile spinal muscular atrophy (ISMA), inclusion body encephalitis and certain dementias (with NINDB-CF, and NCI). To date, the ALS and ISMA material is negative.

Analysis of brain and spinal cord material from ALS and ISMA patients by 10 histochemical reactions disclosed no specific defect in either disease. Prominent astrocytic response in cerebral white matter was demonstrated by oxidative enzyme reactions. Motor neurons and subpial glia showed prominent phosphorylase activity (and slight activity in reactive astrocytes of white matter) in these patients and others, indicating a brisk potentiality for anerobic glycolysis in these cell populations.

The histochemical changes of muscle in ALS was compared and contrasted with changes in other motor neuron diseases and peripheral neuropathies.

## PERIPHERAL NEUROPATHIES

A new neurologic disorder has been reported in two sisters - familial recurrent polyneuropathy in association with alpha-lipoprotein deficiency - Tangier disease (with NHI). This study demonstrates that a metabolic defect can be inherited (parents were partially affected carriers) and constant, yet the clinical disease be intermittent with spontaneous recovery. Delineation of this phenomenon in alpha-lipoprotein deficiency indicates the necessity to renew search for an underlying constant metabolic defect in multiple sclerosis.

Patients with another familial recurrent neuropathy, Refsum's disease, associated with the metabolic defect hyperphytanicacidemia are being quantitatively evaluated clinically (muscle power, motor nerve conduction) to determine whether the low phytol and phytanic acid diet that they were put on by NHI is of any clinical value. Preliminary results suggest the therapy is of some clinical benefit. The ultrastructural changes in peripheral nerve of Refsum's disease is detailed in the Electron Microscopy Project.

A new familial neuromuscular syndrome was demonstrated with the finding of normal plasma alpha and beta lipoproteins (with NHI), in the presence of more than 60% acanthocytes (with NIAMD) in two patients with clinical and histochemical evidence of peripheral neuropathy, slight myopathy, chorea, and seizures. Previously, acanthocytes and neurologic disease have been associated only with beta lipoprotein deficiency (Bassin-Kornsweig disease).

A new syndrome of familial hypertrophic interstitial neuropathy and unusual cataracts has been described.

A new histochemical method of demonstrating the delicate cytoplasmic processes of Schwann cells in single teased nerve fibers has been introduced and applied to events following crush injury of nerve, leading to the hypothesis of a "recoiling phenomenon" of axons.

Target fibers (virtually diagnostic of denervated muscle) were described in the Electron Microscopy Project - their ultrastructural details correlate well with the histochemical results.

Animal studies demonstrated the validity of myosin ATPase as the best histochemical signature of muscle fiber type following experimental denervation, in agreement with the conclusions from study of human disease material. In animals, the ability of nerve to influence muscle was demonstrated in the following ways: (a) conversion of normal newborn guinea pig soleus muscle from mixed to uniform histochemical pattern at 6 weeks - with prevention of that change by neonatal denervation or cordotomy; (b) reversion of adult guinea pig soleus from uniform to mixed histochemical pattern in 6 months following denervation and in only one month following cordotomy; (c) ability of "foreign" nerve fibers to convert muscle fiber types in the guinea pig soleus; and (d) correlation of such histochemically partially converted soleus muscles with changes of physiologic function. "Type grouping" of muscle fibers, demonstrated empirically to be a sign of chronic denervation in humans, was produced experimentally in animals by nerve section and subtotal reinnervation to confirm the hypothesis regarding pathogenesis proposed from the human material.

A new method for recording motor axon potentials in man has been reported.

## OTHER DISORDERS

Contrary to the literature, a case of well documented subacute sclerosing panencephalitis (with elevated measles antibody titers) was shown to undergo marked spontaneous improvement without antiviral or immunosuppressive therapy, antibody titers remaining elevated after improvement (with PRB). In another case of this disorder, normal immunologic defense mechanisms were demonstrated.

Significance to Bio-Medical Research and the Program of the Institute: New details have been accumulated on the morphologic and biochemical substrata of ALS and other diseases of the lower motor neuron. Animal models have been produced of some of these changes. The familial neuropathy, a poorly defined group of disorders, a specific metabolic defect, alpha-lipoprotein deficiency, has been identified for the first time. More accurate evaluation of therapeutic trials are facilitated by (a) development of an instrument for quantitative muscle testing in patients and (b) more complete knowledge of variations in the natural course of the forms of motor neuron diseases.

Proposed Course of Project: To more fully develop studies underway in the hope that identification of metabolic, immune, or infectious etiologies will lead to a means of treating and

preventing these disorders. To apply tissue culture techniques to these problems. To undertake additional therapeutic trials.

Honors and Awards: Three of the following 12 papers were written by invitation.

Publications:

Collis, W.J., and Engel, W.K.: Glucose metabolism in five neuromuscular disorders. Neurology. In Press.

Engel, W.K.: Motor neuron histochemistry in ALS and infantile spinal muscular atrophy. Proceedings of the ALS Symposium Apr/May 1967, San Francisco, California. In Press.

Engel, W.K., and Brooke, M.H.: Muscle biopsy in ALS and other motor neuron diseases. Proceedings of the ALS Symposium Apr/May 1967, San Francisco, California. In Press.

Engel, W.K., Dorman, J., Levy, R.I., and Fredrickson, D.S.: Neuropathy in tangier disease - α-lipoprotein deficiency manifesting a familial recurrent neuropathy and intestinal lipid storage. Arch. Neurol. 17: 1-9, Jul. 1967.

Engel, W.K., Hogenhuis, L.A.H., Collis, W.J., Schalch, D.S., Barlow, M.H., Gold, G.N., and Dorman, J.D.: Metabolic studies and therapeutic trials in amyotrophic lateral sclerosis. Proceedings of the ALS Symposium Apr/May 1967, San Francisco, California. In Press.

Karpati, G., and Engel, W.K.: Correlative histochemical study of skeletal muscle after suprasegmental denervation, peripheral nerve section, and skeletal fixation. Neurology. In Press.

Karpati, G., and Engel, W.K.: Histochemical investigation of fiber type ratios with the myofibrillar ATPase reaction in normal and denervated skeletal muscles of guinea pig. Amer. J. Anat. 122: 145-156, Jan. 1968.

Karpati, G., and Engel, W.K.: Neuronal trophic function - A new aspect demonstrated histochemically in developing soleus muscle. Arch. Neurol. 17: 542-545, Nov. 1967.

Karpati, G., and Engel, W.K.: Type grouping in skeletal muscles after experimental reinnervation. Neurology. In Press.

Karpati, G., and Engel, W.K.: Transformation of the histochemical profile of skeletal muscle by "foreign innervation". Nature 215: 1509-1510, Sep. 1967.

Resnick, J.S., Engel, W.K., and Sever, J.: Subacute sclerosing panencephalitis: Spontaneous improvement in a patient with elevated measles antibody in blood and spinal fluid. New Eng. J. Med. In Press.

Tobin, W.E.: A method for recording motor axon potential in man. J. Appl. Physiol. In Press.

Serial No. NDB(I)-65 MN/OC 1189(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Episodic Weakness

Previous Serial Number: Same

Principal Investigators: Jerome S. Resnick, M.D.
W. King Engel, M.D.

Other Investigators: Robert C. Griggs, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.6 |
| Professional: | 1.6 |
| Other: | 0.0 |

Project Description:

Objectives: To define more clearly those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. Attention is to be directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the neuromuscular system: motor end plate region; sarcolemmal membrane; and sarcolemmal - T system-sarcoplasmic reticulum-myofibrillar complex (excitation contraction coupling mechanism). Further experimentation is to be done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes with a view to obtaining more information regarding pertinent metabolic pathways.

Methods Employed: The techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electron-microscopy, and biochemical study are delineated in other projects. Provocative loading tests and therapeutic trials to raise or lower potassium or sodium are used. Acetazolamide and triamterene were tried as prophylactic agents for hypokalemic periodic paralysis. Electrolyte content and balance were evaluated on and off therapy.

Patient Material: Patients of all ages are admitted to the Medical Neurology Branch for this project if they have intermittent muscular weakness associated with familial periodic paralysis, hypo- or hyperkalemic; isolated examples of periodic paralysis with potassium disturbance; thyrotoxic periodic paralysis; paramyotonia congenita; or myotonia congenita. (Patients with myasthenia gravis are part of another project.)

Major Findings: Acetazolamide appears to be an excellent prophylactic agent effective in 7 of 9 patients with hypokalemic periodic paralysis both sporadic and familial. The patients in whom it is effective have decreased interim weakness, virtually no attacks, and are able to reduce or discontinue their potassium prophylaxis. The mechanism of action is unknown - skeletal muscle is not thought to have carbonic anhydrase, and studies indicate that acetazolamide in these patients does not cause acidosis, reduced serum sodium, or reduced total body exchangeable sodium. Triamterene, a greater natriuric agent, is not an effective prophylactic agent in these patients.

Although it is often stated that the attacks of hypokalemic periodic paralysis are explicable by light-microscopically visible vacuoles in the muscle fibers, our histochemical studies indicate that (a) in patients with moderate or severe attacks there are often far too few or even no vacuoles to account for the weakness, and (b) many vacuoles are present in some clinically normal muscles; hence, another pathogenesis must be considered.

A thyrotoxic form of hypokalemic paralysis has been cured with antithyroid treatment and the concomitant metabolic changes investigated. Reserpine treatment alone reduced the pulse and tremor to normal but did not prevent attacks and interim weakness, whereas propylthiouracil did cure the attacks and the constant between-attack weakness. Hyperaldosteronism was demonstrated before treatment (with NHI) and was normal after treatment. Glucose-insulin loading easily produced attacks of weakness before treatment but not after.

Significance to Bio-Medical Research and the Program of the Institute: The introduction of acetazolamide in the treatment of hypokalemic periodic paralysis appears to have provided the most effective prophylaxis available in this disorder. It benefits about 75% of the patients tested. This new form of prophylaxis provides new avenues for investigation of the basic pathophysiology in this disease. The results in thyrotoxic hypokalemic periodic paralysis suggest epinepherin does not play a role in the weakness, but a secondary hyperaldosteronism has not been ruled out.

Proposed Course of Project: Explore in more detail, with patients and animals, the mechanism of action of acetazolamide prophylaxis in hypokalemic periodic paralysis. Seek even more effective therapeutic agents. Investigate the pathogenesis of thyrotoxic periodic paralysis.

Honors and Awards: None

Publications: Resnick, J.S., Engel, W.K., Griggs, R.C., and Stam, A.: Acetazolamide prophylaxis in hyopkalemic periodic paralysis. New Eng. J. Med. 278: 582-586, Mar. 1968.

Serial No. NDB(I)-65 MN/OC 1190(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Myasthenia Gravis

Previous Serial Number: Same

Principal Investigators: Dale E. McFarlin, M.D.
W. King Engel, M.D.

Other Investigators: John S. Johnson, M.D.
Frederic Q. Vroom, M.D.
Gregory O. Walsh, M.D.
Darryl C. DeVivo, M.D.
Paul W. Hathaway, M.D.

Cooperating Units: Laboratory of Clinical Investigation, NIAID

Man Years:

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|---|---|
| Total: | 1.1 |
| Professional: | 0.6 |
| Other: | 0.5 |

Project Description:

Objectives: To apply histochemical, pharmacologic, electrophysiologic and immunologic techniques to investigate the pathogenesis of myasthenia gravis. Immunologic studies were included because: 1. Immunologic abnormalities have recently been demonstrated in the serum of myasthenic patients. 2. Thymic pathology in patients with myasthenia is well known, and it is now felt that this gland has major immunologic functions.

Methods Employed: Sera from patients with myasthenia gravis were studied by a variety of immunologic techniques, including agar diffusion, immunoelectrophoresis, latex agglutination, and immunofluorescence. Specimens of muscle, and in some cases thymus and thyroid, were studied using immunofluorescent, histochemical and routine histological techniques. The abnormalities were evaluated before and after thymectomy. Clinical studies including prostigmine and curare challenging tests, were done on patients with myasthenia gravis, on a group of patients with myasthenic syndrome in association with

clinical entities with established denervation, and on a number of patients with a variety of other neuromuscular diseases.

Patient Material: Sera, muscle, thymus and other tissue were obtained from Medical Neurology Branch patients.

Major Findings: The immunoglobulins responsible for antinuclear factor and antimuscle factor in serum from myasthenic patients were characterized. Antinuclear factor was found to occur in IgG, IgM and IgA and in some patients was found in all three classes of immunoglobulin. In contrast, antimuscle factor was found to occur only in IgG. This study demonstrated that these two forms of reactivity behave as separate entities.

Treatment of adult myasthenics with short term ACTH has produced improvement in the few cases in which this has been tried. It is noteworthy that a striking improvement in a patient with juvenile myasthenia gravis after receiving long term ACTH treatment was not preceded by weakness which is usually seen in adult patients with myasthenia gravis.

Studies in curare sensitivity at 2.5 - 10 μgm/Kgm body weight continue to reveal that this phenomenon is specific for myasthenia gravis, in our hands.

Significance to Bio-Medical Research and the Program of the Institute: The demonstration that the antimuscle factor in patients with myasthenia gravis does not differ immunochemically from the antimuscle factor in patients with thymoma and no myasthenia gravis supports our suspicion that this agent per se is not responsible for the neuromuscle block in this disease. The observation that antimuscle factor and antinuclear factor behave as separate serological entities raises the possibility of a third as yet undescribed serological abnormality which is the agent responsible for the defect in this condition. The response of myasthenic patients to ACTH indicates that much of the abnormality is not irreversible and supports the idea that improvement can result even in the most severely affected and advanced stages of this disease.

Curare-testing continues to be an important diagnostic tool for differentiating myasthenia gravis from other neuromuscular disorders.

Proposed Course of Project: In selected patients, specific aspects of this disease will be investigated: post-thymectomy state, neonatal myasthenia, mechanism of ACTH treatment, and newer forms of therapy. Techniques involving the study of cellular

immunity are to be added to the investigative program, which should facilitate characterization of the thymic abnormality in myasthenia gravis.

Honors and Awards: None

Publications: McFarlin, D.E.: Serological abnormalities in myasthenia gravis. Neurology 18: 274, Mar. 1968.

McFarlin, D.E., Johnson, J.S., and Seymour, W.F.K.: Antimuscle factor and antinuclear factor in patients with myasthenia gravis. I. Heavy chain determinants. J. Immunol. In Press.

Griggs, R.C., McFarlin, D.E., and Engel, W.K.: Severe occult juvenile myasthenia gravis responsive to long-term corticosteroid therapy. Trans. Amer. Neurol. Ass. In Press.

Serial No. NDB(I)-65 MN/OC 1191(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Immunological Abnormalities of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: Dale E. McFarlin, M.D.
Robert C. Griggs, M.D.

Other Investigators: Thomas A. Waldmann, M.D.
Warren Strober, M.D.
Joost Oppenheim, M.D.
John S. Johnson, M.D.
Darryl C. DeVivo, M.D.

Cooperating Units: Metabolism Branch, NCI
Medicine Branch, NCI
Human Genetics Branch, NIDR
Laboratory of Clinical Investigation, NIAID

Man Years:

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|---|---|
| Total: | 2.0 |
| Professional: | 1.5 |
| Other: | 0.5 |

Project Description:

Objectives: To apply immunological techniques to the study of diseases affecting the central nervous system and neuromuscular system.

Methods Employed: Standard immunochemical techniques were employed. In addition, preliminary use of tissue culture techniques was begun, especially to study lymphocyte transformation.

Patient Material: Specimens of peripheral blood, bone marrow, muscle, peripheral nerve, thymus and other tissue were obtained from patients with neurological disease admitted to the Medical Neurology Branch. Serum and lymphocytes from these patients were also provided to the Pediatric Neurology Section, Surgical Neurology Branch.

Major Findings: 1. In ataxia telangiectasia the following were found: (a) In two additional patients the defect of immunoglobulin-A (IgA) synthesis was confirmed. (b) In four additional patients defective lymphocyte function was documented; the mechanisms responsible for this defect are currently being investigated. (c) Glucose metabolism was found to be normal in two patients which is contrary to our past experience with this disease.

2. Four patients with evidence of inflammatory myopathies have been treated with an immunosuppressive drug, azathioprine; dramatic improvement was documented in three patients and a suggestion of improvement was seen in the fourth patient.

3. Immunological evaluation of a patient with subacute sclerosing panencephalitis disclosed normal immunologic mechanisms.

4. Specimens collected in the serum and spinal fluid bank have proven to be valuable in the study of a number of entities, possibly of viral and/or autoimmune etiology.

Significance to Bio-Medical Research and the Program of the Institute: Elucidation of the defects responsible for abnormalities in ataxia telangiectasia may lead to learning the mechanisms responsible for the nervous system pathology. Whether the underlying immunological defect present in ataxia telangiectasia predisposes to an infection ultimately responsible for the central nervous system disease remains to be seen. Viral studies on our patients have thus far been negative. The mechanism in this disease, when elucidated, may have direct bearing on the pathogenesis of multiple sclerosis.

On the basis of our pilot study, additional trial of immunosuppressive therapy of the inflammatory myopathies (polymyositis) is indicated. In addition to the therapeutic connotations of our study, there are implications concerning the pathogenesis of inflammatory myopathies. Since azathioprine clearly produced remission, it may prove of value for delineating the pathogenesis of inflammatory myopathies.

Proposed Course of Project: The immunological defects in ataxia telangiectasia are to be categorized more fully with the addition of the latest immunological techniques. Additional studies of cellular immunity in a number of neurological conditions are planned to be started when additional space and necessary equipment become available.

Honors and Awards: None

Publications:

McFarlin, D.E., and Griggs, R.C.: Treatment of inflammatory myopathies with azathioprine. Trans. Amer. Neurol. Ass. In Press.

Strober, W., Wochner, R.D., Barlow, M.H., McFarlin, D.E., and Waldmann, T.A.: Immunoglobulin metabolism in ataxia telangiectasia. J. Clin. Invest. In Press.

Strober, W., Wochner, R.D., McFarlin, D.E., Barlow, M.H., and Waldmann, T.A.: Immunoglobulin A metabolism in ataxia telangiectasia. Clin. Res. 15: 300, 1967. Abstract.

Wong, V.G., and McFarlin, Dale E.: Primary familial amyloidosis. Arch Ophthal. 78: 208, 1967.

Serial No. NDB(I)-65 MN/OC 1192(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electron Microscopic Studies of Skeletal Muscle and Neurons

Previous Serial Number: Same

Principal Investigators: Jerome S. Resnick, M.D.
W. King Engel, M.D.
Michel Fardeau, M.D.

Other Investigators: William H. Olson, M.D.

Cooperating Units: None

Man Years

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|---|---|
| Total: | 1.1 |
| Professional: | 0.6 |
| Other: | 0.5 |

Project Description:

Objectives: To study the early fine cellular changes of human muscle in diseases that are confined to muscle alone, as well as in conditions which produce secondary alterations in muscle such as denervating and metabolic diseases. To study fine cellular changes in certain diseases affecting neurons.

Methods Employed: A portion of biopsied muscle is tied to a glass rod to maintain resting length and immediately fixed in cold buffered osmium tetroxide for araldite embedding. Various EM staining procedures are used. Adjacent biopsy material is immediately frozen for cryostat sections and incubated in various histochemical media, as described in another project. Some stained cryostat sections are ready within an hour after the biopsy. These sections can be used as a guide to determine which of the biopsies contain light microscopic features of special interest and thereby serve as a guide for choosing material appropriate for electron microscopic study. Biopsy specimens of human nerve or brain tissue are fixed and embedded for electron-microscopy and are similarly controlled by simultaneous enzyme histochemistry.

Patient Material: Patients with various myopathies and neurogenic muscular weakness are the major sources of material. Congenital non-progressive neuromuscular dysfunction without evident pathologic changes on light microscopy and histochemistry (so-called benign congenital hypotonia) are also under study. When available, tissue from asymptomatic carriers of progressive genetic myopathies is taken for study. Occasional brain biopsies are obtained from patients with certain forms of progressive degenerative disease.

Major Findings: Target fibers were first identified from this Branch in 1961 by use of histochemical techniques and were considered a sign of denervation. Now the ultrastructural changes of target fibers have been reported and correlated with histochemical changes in the same biopsy specimen. The central zone shows absence of mitochondria and reticulum, severely reduced aqueous sarcoplasm (including glycogen granules), and loss of myofibrillar organization. Myofibrillar material is present, but the filaments are not in neat transverse arrays and there appears to be excessive dark, Z-band-like substance smeared irregularly throughout the sarcomere. The intermediate zone often has increased amounts of mitochondria, reticulum, and glycogen. These findings demonstrate that target fibers differ from fibers in central core disease only quantitatively (not qualitatively), so that rather than being a "myopathy", central core disease may be a result of defective motor innervation, as postulated earlier from this Branch on the basis of histochemical studies of that disease.

The EM proof of periodicity and Z-band origin of rods experimentally produced in cat by tenotomy was obtained, indicating their identity with rods developing spontaneously in the human disease rod (or nemaline) myopathy (see Project "Myopathies").

Details of mitochondrial abnormalities - enlargement, amorphous lipid-like inclusions, organized box-car-like inclusions between the inner and outer membrane, and contorted cristae - have been elucidated in three unusual kinds of muscle disease.

What were initially described from this Branch on the basis of histochemical studies as "mitochondrial aggregates" have now been found by EM to be "tubular aggregates", apparently collections of proliferated sarcoplasmic reticulum.

Muscle fibers in the new disease "Type I fiber hypotrophy with internal nuclei" reported from this Branch have been described electron-microscopically.

In Refsum's disease (atactica hereditaria polyneuritiformis), which has a concomitant hyperphytanicacidemia, the neuropathy was

shown to be primarily a defect of Schwann cells, those cells showing (a) marked hypertrophy, and (b) very distinctive "crystalline" inclusions in mitochondria.

An EM search for viral particles in polymyositis and other sporadic neuromuscular diseases has been initiated.

Significance to Bio-Medical Research and the Program of the Institute: Analysis of the changes in ultrastructure of the neuromuscular disorders is in its infancy. Identification of new forms of disease occur rapidly in this stage. However one must be careful not to name a condition on the basis of careful though limited EM or histochemical studies. Therefore, a broad experience with as many well-studied cases as possible is necessary, as is broad correlation of EM with histochemistry. It appears that certain morphologic changes can indicate suspected metabolic defects, not necessarily "primary", as in contractile protein defects in rod myopathy and in "tubular aggregates". Electron microscopy can identify similar ultrastructural changes in clinically dissimilar disorders, e.g. central core disease and peripheral neuropathy with target fibers, and vice-versa. These ultrastructural changes will help guide formulation of pathogenesis and treatment of the neuromuscular and neurologic disorders. Experimental production of identical defects, e.g. rods in animals, confirmed by EM, provides a new tool for analysing these changes. Similar expectations pertain to the other human neuromuscular disorders being studied, as well as the neuronal abnormalities. The substance composing the "crystalline" inclusions in Refsum's disease may provide further clues of the pathogenesis of this disease.

Proposed Course of Project: Further studies that combine histochemical, immunologic, autoradiographic and tissue culture techniques with electron-microscopy applied to human neurologic diseases and animal models have been planned, so as to supplement the morphologic approach with pertinent studies of cellular dynamics.

Honors and Awards: One paper was written by invitation.

Publications: Resnick, J.S., and Engel, W.K.: Target fibers - Structural and cytochemical characteristics and their relationship to neurogenic muscle disease and fiber types. In Milhorat, A.T. (Ed.): Exploratory concepts in Muscular Dystrophy and Related Disorders. New York, Excerpta Medica Foundation, 1967, pp. 255-266.

Resnick, J.S., Engel, W.K., and Nelson, P.G.: Changes in the Z disk of skeletal muscle induced by tenotomy. Neurology. In Press.

Serial No. NDB(I)-65 MN/OC 1193(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Radioautography Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: David E. Pleasure, M.D.
W. King Engel, M.D.

Other Investigators: Jon D. Dorman, M.D.

Cooperating Units: None

Man Years:

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|---|---|
| Total: | 2.0 |
| Professional: | 1.0 |
| Other: | 1.0 |

Project Description:

Objectives: To apply radioautographic techniques with use of nucleic acid, protein, and lipid precursors to the study of diseases affecting the nervous system and/or skeletal muscles. To obtain baseline animal studies in normal and experimental states. To determine sensory and motor axonal flow rates in normal and neuropathic animals with tritiated leucine.

Methods Employed: Radioautography was employed utilizing the excellent resolution afforded by the low energy β-radiation of tritiated compounds; Kodak NTB 2 emulsion was used for processing the tissue with the dipping technique of Leblond and Kopriwa. Tissues from the central and peripheral nervous system and muscle were studied, using tritiated uridine, leucine, or palmatate.

Patient Material: During this year, patient material was not utilized.

Major Findings: Study of the uptake pattern of the intracysternally administered labeled uridine in different parts of the CNS of a series of rabbits had revealed a spatiotemporal

optimum of one hour for uptake in the nucleus of large neurons and an optimum of two days for uptake in the cytoplasm of the large neurons.

Axonal flow rates, both motor and proximal and distal sensory, determined in 32 cats, 16 normal and 16 with experimental axonal neuropathies (acrylamide, triorthocresyl phosphate) have disclosed: (a) proximal and distal flow rates from dorsal root ganglia are symmetric both in speed and in quantity. In normal cats, sensory flow rates range from 1.1 to 1.7 millimeters/day. (b) there is a slight slowing of sensory axonal flow in diseases of the axon, particularly acrylamide neuritis. (c) motor flow rates in normals is of the same magnitude as sensory.

Preliminary results suggest a differential uptake and utilization of tritiated fatty acids between Type I and Type II skeletal muscle fibers.

Significance to Bio-Medical Research and the Program of the Institute: The kinetics of uridine metabolism in rabbit neurons are the basis for similar studies in humans. The determination of axonal flow rates in diseases of the lower motor neuron offers a new avenue of approach to an understanding of their pathogenesis. In particular, we hope to be able to distinguish between lesions primarily of the cell perikaryon and those specifically of the axon.

Proposed Course of Project: Planned extensions of our study of axonal flow include analysis of axonal flow rates in demyelinating neuropathies, diphtheritic and experimental allergic. We hope to extend our study to uptake of tritiated fatty acids by muscle to human biopsy material and to use other metabolic precursors. We will then be able to learn whether diseases primarily affecting one or the other muscle fiber type (e.g. myotonic dystrophy - Type I fibers) are associated with abnormalities in substrate uptake or utilization.

Honors and Awards: None

Publications: Hogenhuis, L.A.H. and Spaulding, S.W.: Autoradiography of long-term RNA metabolism in rabbit neurones. Nature 215: 281-283, Jul. 1967.

Serial No. NDB(I)-62 MN/NR 925(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Small Sella Turcica

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Thomas Aceto, Jr.
Mary Parker
Alvin Hayles
Colin B. Holman

Other Investigators: None

Cooperating Units: The National Pituitary Agency, Baltimore, Maryland

The Children's Hospital, Buffalo, New York

Washington University, St. Louis, Missouri

The Mayo Clinic, Rochester, Minnesota

Man Years:

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|---|---|
| Total: | .1 |
| Professional: | .1 |
| Others: | 0 |

Project Description:

Objectives: An anatomo-radiographic study to confirm in a large series of patients the diagnostic significance of the small sella turcica. In addition, we plan to establish whether or not the sella turcica of hypopituitaric dwarfs changes in size after growth hormone treatment.

Methods Employed: Volumetric studies of the sella turcica in many hypopituitaric dwarfs before and after growth hormone treatment.

Major Findings: During last year many more sella turcica measurements of dwarfs have been gathered. A group of these patients have already been checked as far as possible changes of the sella turcica size after a first treatment course with growth hormone.

Significance to Bio-Medical Research and the Program of the Institute: The rarity of the small sella turcica in the average hospital population and in non-hypopituitaric dwarfs and the frequency of this radiographic finding in hypopituitaric dwarfs make this x-ray sign a valuable diagnostic element. The diagnosis of idiopathic hypopituitaric dwarfism in pre-pubertal children is difficult and dependent to a large degree upon indirect chemical estimations of various trophic hormone deficiencies of the pituitary. The finding of a sella volume below the normal range for the patient's age should suggest and should be considered as confirmatory evidence of hypopituitarism in the doubtful cases.

Proposed Course of Project: Evaluation of the gathered data, in particular comparison of the sella turcica measurements before and after treatment with growth hormone.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-63 MN/NR 1047(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Isotope-Ventriculography and Isotope-Cisternography

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro

Other Investigators: William L. Ashburn
William H. Briner
Ayub K. Ommaya

Cooperating Units: Department of Nuclear Medicine, Diagnostic Radioisotope Section, Clinical Center, NIH

Radiopharmaceutical Service, Pharmacy Department, Clinical Center, NIH

Surgical Neurology Branch, National Institute of Neurological Diseases and Blindness, NIH

Man Years:

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|---|---|
| Total: | .3 |
| Professional: | .3 |
| Others: | 0 |

Project Description:

Objectives: A gamma emitting isotope injected within the cerebrospinal fluid pathways will permit in subsequent head scans the pictorial outline of the ventricular system (isotope-ventriculography) and of the subarachnoid intracranial spaces (isotope-cisternography). Information about the anatomical status of the cerebrospinal fluid cavities, and, by multiple serial scans, of the normal and abnormal dynamics of the cerebrospinal fluid itself will be obtained.

Methods Employed: We have now expanded the choice of the tracers for isotope-ventriculography and isotope-cisternography. Besides low protein RIHSA and $^{99m}TcO_4^-$, we have introduced high specific activity $^{99m}$Tc-HSA. High specific activity tagging of the albumin (up to 12 millicuries per milligram of albumin) has been accomplished by Mr. William H. Briner of our Radiopharmaceutical Service. This new tracer has the following advantages:

1) favorable physical characteristics of $^{99m}Tc$; 2) "advantageous" biological behavior of the albumin; 3) lower critical organ (spinal cord and brain) and total body radiation; 4) possibility of effective and efficient use of the gamma scintillation camera. The use of this instrument, recently acquired by the NIH, represents the other important recent improvement in isotope-cisternography. The combination of $^{99m}Tc$-HSA and the camera has allowed us to study more efficiently and accurately the cerebrospinal fluid rhinorrheas. We may actually "photograph" the tagged cerebrospinal fluid while it runs out from the subarachnoidal spaces into the nasal cavities. High specific activity RIHSA has not lost its importance as a tracer for isotope-cisternography, in particular in cases where late (24, 48, and 72 hours) records are necessary. In a certain number of patients both $^{99m}Tc$-HSA and RIHSA have been introduced intra-thecally, giving us the opportunity to have early (30 minutes, 1 hour, 2 hours) records ($^{99m}Tc$-HSA) and late (24, 48, 72 hours) records (RIHSA). The discrimination allowed by pulse height analysis has permitted this double tracer technique. $^{99m}TcO_4^-$ remains the tracer of choice for most of the ventriculographies and the study of the corrective surgical shunts.

Major Findings: The techniques of isotope-ventriculography and, in particular, isotope-cisternography are now well established, routine diagnostic procedures. Hundreds of patients have been studied by these techniques. Traumatic and "spontaneous" cerebro-spinal fluid leaks, leptomeningeal cysts, non-communicating hydrocephalus due to arachnoiditis or subarachnoidal bleedings, the porencephalic cysts and evaluation of neurosurgical shunts remain the main indications of these diagnostic methods. The successful surgery in patients (previously operated on "blindly" and, therefore, often without success) with cerebrospinal fluid leaks and in patients with leptomeningeal cysts and CSF pathways obstructions has been possible because of the diagnostic information obtained with these radioactive scanning procedures.

Significance to Bio-Medical Research and the Program of the Institute: Legions of authors are studying this remarkable fluid (CSF) which still remains uncomprehended since Cotugno first described it in 1764. In particular, we have now a diagnostic tool to gather information about the "terra incognita" which is represented by the basal and convexity subarachnoidal pathways.

Proposed Course of Project: Further information about the normal and abnormal cerebrospinal fluid cavities, and the normal and pathologic flow of the CSF will be gathered by the techniques of isotope-cisternography and isotope-ventriculography.

Particular attention will be devoted to the study of the various types of hydrocephalus. The techniques of isotope-cisternography and isotope-ventriculography are ideally suited to study the physiopathology of hydrocephalus. From the pragmatic point of view, these isotopic techniques are the best available for deciding the types of hydrocephalus which should be treated with the different types of CSF shunting. We plan to gather further experience in this field so that definite guidelines for the treatment of hydrocephalus will be established.

Honors and Awards: None

Publications:

Di Chiro, G., Ommaya, A. K., Ashburn, W. L., Briner, W. H.: Isotope cisternography in the diagnosis and follow-up of cerebrospinal fluid rhinorrhea. J. Neurosurg. (In press).

Di Chiro, G., Ashburn, W. L., and Briner, W. H.: High specific activity $^{99m}$Tc-albumin as a tracer for subarachnoidal and ventricular scintiphotography. Arch. Neurol. (In press).

Serial No. NDB(I)-65 MN/NR 1195(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Selective Arteriography of Spinal Cord Arteriovenous Aneurysms

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
John L. Doppman
Ayub K. Ommaya

Other Investigators: None

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Surgical Neurology Branch, National Institute of Neurological Diseases and Blindness, NIH

Man Years:

| | |
|---|---|
| Total: | .3 |
| Professional: | .3 |
| Others: | 0 |

Project Description:

Objectives: Diagnosis of the nature of space-occupying lesions within the spinal canal is often difficult. In most instances with myelography, we are only able to put in evidence a total or partial subarachnoidal block. Myelograms, on the other hand, are seldom helpful in establishing the type of lesion which has caused the block. With recent advances in the field of arteriography--safer radiographic contrast media, better needles, and better catheters, and the revival of interest in subtraction--we feel that a systematic angiographic evaluation of the intraspinal mass lesions is worthwhile. Angiography is of particular importance, of course, in spinal cord vascular malformations. Significant information might, however, be obtained in other types of lesions, especially spinal cord tumors. It is conceivable that tumor, pathologic vessels, and displacement of the large spinal cord arteries (in particular, the arteria radicularis magna--Adamkiewicz) may be demonstrated in cases of spinal cord tumors in analogy to similar angiographic findings in brain tumors.

Methods Employed: Selective arteriograms with the use of modern catheter techniques are carried out in patients in whom intraspinal space-occupying lesions are suspected. The subtraction technique of Ziedses des Plantes is used to better visualize the injected vessels. Recently, however, we have put much less emphasis on subtraction because we are now convinced that the crucial aspect of this technique is the selectivity of the injection.

Major Findings: Selective arteriography of the spinal cord is now established as the diagnostic procedure of choice in cases of arteriovenous malformations of the spinal cord. Angiographic demonstration of the arteries feeding these malformations has been possible in fifteen cases. The diagnostic applications of selective arteriography of the spinal cord vessels has been extended to other pathological conditions. Thus, displacement of the arteria radiculo-medullaris magna (Adamkiewicz) and the anterior spinal artery has been observed in five patients with space-occupying lesions (tumors and others) in and around the spinal cord. This displacement, analogous to the one of the cerebral vessels in cerebral angiography, has made possible, in one case, the identification of the upper limit of a cord tumor, limit which had not been recognized by means of other radiodiagnostic studies (myelography). Arteriography of the spinal cord has also allowed the visualization "in vivo" of collateral flow through dilated spinal cord arteries ("the hypertrophied spinal artery syndrome") in association with aortic coarctation and extraspinal arteriovenous shunts. The recognition of these dilated spinal cord arteries is important because these engorged vessels may be responsible for cord compression, and if they rupture, for spinal subarachnoid bleedings. Finally, the possibility of consistently demonstrating the artery of Adamkiewicz (main blood supplier of the thoraco-lumbar cord) by the technique of selective arteriography furnishes the surgeon undertaking any procedure involving the descending thoracic and upper abdominal aorta, with valuable information concerning the cord vascularization. This information may be helpful to prevent the much dreaded spinal cord damage after aortic surgery.

Significance to Bio-Medical Research and the Program of the Institute: Selective arteriography of the spinal cord will undoubtedly increase our understanding of the large group of the conditions in which vascular lesions of the cord represent the basic pathologic element.

Proposed Course of Project: Further technical improvement of the selective arteriography of the spinal cord technique. We are particularly interested in radiographic magnification using the new fractional focal spots x-ray tubes. The resolution of the small vessels should be improved with these magnification techniques.

We have already started spinal cord angiography in monkeys to be used as an experimental model.

We are planning to increase our clinical angiographic experience.

We are also planning angiographies of spinal cord vessels in cadavers, with intravascular injection of some new types of plastics, followed by corrosion of the specimen (so that the vessels will stand out in all their detail).

Honors and Awards: None

Publications:

Di Chiro, G., Doppman, J., and Ommaya, A. K.: Selective Arteriography of Arteriovenous Aneurysms of Spinal Cord. Radiology 88:1065-1077, 1967.

Doppman, J. L., Di Chiro, G., and Ommaya, A. K.: Selective Arteriography of Spinal Cord Vessels with particular regard to Arteriovenous Aneurysms. St. Louis, Missouri, Warren H. Green, Publisher, 1968. In press.

Serial No. NDB(I)-65 MN/NR 1196(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Clinical Comparison of Radioactive Tracers Used for Brain Scanning

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
William L. Ashburn
Arthur S. Grove, Jr.

Other Investigator: William H. Briner

Cooperating Units: Department of Nuclear Medicine, Diagnostic Radioisotope Section, Clinical Center, NIH

Radiation Safety Department, Clinical Center, NIH

Radiopharmaceutical Service, Pharmacy Department, Clinical Center, NIH

Man Years:

| | |
|---|---|
| Total: | .2 |
| Professional: | .2 |
| Others: | 0 |

Project Description:

Objectives: A variety of isotopes are now being used for brain scanning. Multiple scans with multiple isotopes carried out in the same patients provide the best method for comparatively appraising the various scanning agents. Few studies of this type have been reported, and we hope that by scanning a large number of patients we can provide information about selective uptake of tracers which has been previously unavailable.

Methods Employed: RIHSA (radio-iodinated human serum albumin), $^{99m}TcO_4$ (technetium pertechnetate), $^{197-203}Hg$ neohydrin (mercury neohydrin), and RIAF (radio-iodinated antifibrinogen) are the tracers used. In addition, in the last part of 1966, we introduced a new tracer: $^{99m}Tc$-HSA (technetium human serum albumin). The comparison between the various tracers is done

by injecting multiple tracers in the same patient. The two basic facts which allow us to carry out this study are the different half-life of the various isotopes and the use of pulse height analysis. Most patients receive three of the five above-mentioned tracers. Sometimes four and exceptionally five tracers are used in the same patient. The NIH Tetrascanner is used for most of these studies and in a significant number of instances we are also employing the gamma scintillation camera of the Anger type.

Major Findings: a) Most space-occupying intracranial lesions can be diagnosed by several isotopes, but in some cases a diagnosis can be reached only or much better with one tracer; b) RIHSA has a "relative" specificity for metastatic lesions; c) $^{197}$Hg neohydrin has a "relative" specificity for all glial tumors; d) RIAF may have specificity for sarcomas and certain large clots; e) $^{99m}TcO_4^-$ is probably most useful as a quick screening tracer and it can be most effectively employed with an Anger-type camera; f) Judging from our preliminary experience, $^{99m}$Tc-HSA looks very promising. It combines the biological advantages of albumin with the favorable physical characteristics of $^{99m}$Tc. Several cerebral lesions not demonstrated with other tracers were put in evidence with $^{99m}$Tc-HSA. With this compound the radiation dosage to the patient is markedly reduced. Furthermore, when using $^{99m}$Tc-HSA, one may effectively and efficiently employ both a rectilinear scanner and a scintillation camera of the Anger type.

Significance to Bio-Medical Research and the Program of the Institute: It is evident that although the majority of space-occupying intracranial lesions can be demonstrated by using any one of the several available brain scanning tracers, certain lesions may be demonstrated only or much more clearly by a specific tracer. Whenever there is strong clinical or neuroradiologic evidence of an intracranial space-occupying lesion, multiple tracer studies are, therefore, indicated because of the possible increase in information yield. The radiation dosage received by the patient, although an important consideration, should not dissuade the clinician from obtaining additional data concerning the nature and location of the lesion. Diagnostic "screening" by scanning is, of course, a different matter and here $^{99m}TcO_4^-$ probably remains the single most favored tracer. Nonetheless, we must conclude from our own experience that when more than one tracer is employed, the accuracy of the scanning procedure increases. Whenever the results of a brain scan with one tracer are equivocal, a repeat scan using a different tracer is indicated and would appear to be preferable to merely repeating the scan with the original tracer. Finally, additonal experience is needed to assess whether or not a newly introduced agent, $^{99m}$tc-HSA, which theoretically has several desirable qualities, should become a frequent choice for brain scanning.

Proposed Course of Project: Completed.

Honors and Awards: None

Publications: Di Chiro, G., Ashburn, W. L., and Grove, A. S.: Which Radioisotopes For Brain Scanning? Neurology 18:225-236, 1968.

Serial No. NDB(I)-66 MN/NR 1283(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Empty Sella Turcica

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro

Other Investigators: None

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Surgical Neurology Branch, National Institute of Neurological Diseases and Blindness, NIH

Endocrinology Branch, National Cancer Institute, NIH

Pathologic Anatomy Branch, National Cancer Institute, NIH

Man Years:

| | |
|---|---|
| Total: | .1 |
| Professional: | .1 |
| Others: | 0 |

Project Description:

Objectives: During previous anatomic work on the sella turcica, "empty" sellae, i.e. instances in which the pituitary may so far fail to fill the sella that the anatomic specimen appears empty, were observed in several cases. The empty sella is usually larger than normal: In fact, in a series of 66 postmortem anatomic observations of the sella turcica and its content the largest sella was an "empty" one. A selective hydrocephalus of the intrasellar subarachnoid space is considered to be the cause of the large empty sella.

The existence of a large empty sella has pragmatic importance. A large empty sella may mimic an intrasellar tumor and a differential diagnosis has to be made. In addition, the abnormal dilatation of the intrasellar subarachnoid space may cause, probably with a pressure mechanism, a hypopituitaric syndrome

(as in several of our cases) or the development of an intrasellar tension cyst. These cysts may have eroding ability resulting in a break of the sellar floor and an ensuing cerebrospinal fluid rhinorrhea. The empty sella acquires, therefore, significance as a specific pathologic entity. Finally, the knowledge of the existence of a large intrasellar subarachnoid space is important if therapy of the pituitary gland (whether trans-sphenoidal hypophysectomy, trans-sphenoidal $^{90}Y$ interstitial therapy, or external irradiation) is contemplated.

Methods Employed: Suitable views (hanging-head lateral with autotomography or zonography) are taken at the end of most pneumoencephalographies. Prior to taking these special views the patient is kept in the hanging-head position for at least five minutes, the intent being to fill with air the intrasellar subarachnoid space if present. Particular attention is paid to patients with large sellas and with "dyspituitarism". Occasionally surgical or autopsy follow-up is obtained.

Major Findings: Moderately large to large or very large intrasellar subarachnoid space (cisterna intrasellaris) has been observed in an unexpectedly high number of patients with normal, borderline big, or big sellas. The intrasellar subarachnoid space is located in the anterior part of the sella, with the pituitary gland occupying the infero-posterior part of the sellar cavity. In certain instances the compressed pituitary gland appears to be reduced to a shell-like structure filling only the postero-inferior section of the sella.

Significance to Bio-Medical Research and the Program of the Institute: The knowledge of the large empty sella is important for: a) the differential diagnosis of intrasellar tumor; b) the explanation of certain hypopituitaric syndromes and certain types of spontaneous cerebrospinal fluid rhinorrhea; and c) to avoid mistakes when contemplating sphenoidal (surgical and $^{90}Y$) or external radiation pituitary treatment.

Proposed Course of Project: The gathered material needs to be tabulated and evaluated so that the precise meaning of the empty sella turcica in normal and pathological conditions can be established.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-66 MN/NR 1284(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Tetrascanner

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
William L. Ashburn

Other Investigators: None

Cooperating Units: Department of Nuclear Medicine, Diagnostic Radioisotope Section, Clinical Center, NIH

Man Years:

| | |
|---|---|
| Total: | .1 |
| Professional: | .1 |
| Others: | 0 |

Project Description:

Objectives: To compare the usefulness of a Rectilinear scanner (in particular the NIH Tetrascanner) versus the gamma camera (Anger type) in the field of brain scanning in its different applications (conventional brain scanning, isotope cisternography, isotope ventriculography, etc).

Methods Employed: A Gamma scintillation camera of the Anger type has been available since February, 1967 at the NIH. Most of the patients have been studied since then, using both the rectilinear scanning (Tetrascanner) and the camera.

Major Findings: The rectilinear scanner and the camera are not exclusive of each other but rather complementary. The camera is ideally suited for studies in which speed is of paramount importance, as emergency examinations of patients in coma or in noncooperative patients unable to remain immobile for a long time. A typical example of an advantageous application of the camera is the study of the cerebrospinal fluid rhinorrheas: Here we are able, due to the speed of operation of the camera, to position the patient so that the CSF fluid runs out from the nose and "photograph" the radioactively "hot"

fluid while it is actually leaking. Thus, the leaking trail may be directly demonstrated. When, however, detailed demonstration of the endocranial structures is indispensable, the higher resolution of the rectilinear scanner is very advantageous. One typical example of the advantage of using a rectilinear scanner is represented by certain cases of normotensive hydrocephalus where, in the camera scintiphotograms, it is sometimes difficult or not possible to decide the important point whether or not the tracer has penetrated into the ventricular system. The rectilinear scanner records, because of their higher resolution, allow to recognize the intraventricular penetration.

Obviously, the Tetrascanner with its four detecting probes, is a particular type of rectilinear scanner. By allowing four simultaneous views, it permits a much faster examination than it is possible with a single probe rectilinear scanner. Thus, the Tetrascanner, by combining the favorable features of the rectilinear scanners (resolution) and of the cameras (speed) may indicate the way to the future development of the scanning devices.

Significance to Bio-Medical Research and the Program of the Institute: The two classes of detecting devices used for localization of radioactivity in vivo, that is rectilinear scanners and cameras, are complementary to each other. In every department of nuclear medicine, a rectilinear scanner and a camera should be available. Multiple head moving-detector scanners (as the NIH Tetrascanner) may represent the desirable future approach to brain scanning. With multiple probes scanners speed is gained, while at the same time resolution is preserved.

Proposed Course of Project: This project is completed.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 MN/NR 1413(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Radioactive Scanning Tomography of the Brain

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Floro Miraldi

Other Investigators: None

Cooperating Units: Department of Nuclear Engineering
Case Western Reserve
Cleveland, Ohio

Man Years:

| | |
|---|---|
| Total: | .1 |
| Professional: | .1 |
| Others: | 0 |

Project Description:

Objectives: Radioisotopic brain scanning is fast becoming one of the essential diagnostic methods to explore the brain. Its reliability in detection and localization of intracranial space-occupying lesions is high (about 80%) but still somewhat lower than that of other neuroradiologic procedures (cerebral angiography, pneumoencephalography). To improve the accuracy of brain scanning, we may essentially follow two research avenues: 1) better tracers; 2) improvement of the equipment. The improvement of the equipment is aimed at increasing the resolution without a substantial loss of sensitivity. This double goal will undoubtedly be reached if a method of layer body section (tomography) associated with scanning can be developed.

Methods Employed: A radioactive brain scan (obtained with a rectilinear scanner) or a scintiphoto (obtained with a fixed device such as a gamma scintillation camera) represents a complex record of signals (photons) originating from the endocranial, cranial and facial tissues. All these signals superimpose in a pattern in which it is often difficult to discriminate between the various anatomical elements. An improved analysis of the scintillographic record of the head would be attained by tomography.

We are at present theoretically analyzing the various methods (swinging detector, dome-like crystal, "focal plane" of the positron camera, multiple "crossing" focusing detectors, positrons "time of flight") tried by other investigators to solve the problem of scanning tomography. We are also considering new approaches. A mathematic appraisal of the three fundamental parameters--sensitivity, resolution, and efficiency--represents the Phase I in our approach to the problem of scanning tomography.

Major Findings: A mathematical analysis of the three fundamental parameters--sensitivity, resolution, and efficiency--of the various devices (already available or proposed as possibilities) for scanning tomography, is well under way with the help of a computer.

Significance to Bio-Medical Research and the Program of the Institute: A successful and simple method of scanning tomography would revolutionize the field of neuroradiology, and parenthetically other fields of radiology. Brain scanning and brain scintiphotography are in fact essentially innocuous (no mortality or morbidity), causing no discomfort to the patient, "less radiation giving" (if proper isotopes are used), and cheaper than other neuroradiologic procedures. Brain scanning and brain scintiphotography are also simple, quick procedures and may in fact, in most instances, be carried out on an ambulatory basis without admitting the patient to the hospital. Last but not least, interpreting a brain scan or a scintiphoto is much simpler and straight-forward than reading a cerebral angiogram or a pneumoencephalogram. If, therefore, the resolution of brain scanning can be significantly improved by tomography, a complete change-over of the neuroradiology departments will occur. Brain scanning and brain scintiphotography would acquire further diagnostic importance for the detection of intracranial tumors, bleedings, and infarctions following strokes. Some of these lesions--especially the ones of the infiltrating type--which today escape detection by any of our diagnostic neuroradiologic methods, would then probably be "picked up". An increase, in absolute terms, of the detecting accuracy of the neuroradiologic methods taken as a "whole" would then occur.

Proposed Course of Project: When the mathematical analysis of the pertinent parameters concerning the various scanning tomography devices is completed, we will be able to appraise the relative merits and pitfalls of the various methods: Perhaps we will then proceed to the Phase II, that is building a Prototype Tomoscanner.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 MN/NR 1414(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Angiographic and Pathologic Aspects of Radiation Arteritis of the Brain in Humans

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Daniel W. Bruce

Other Investigators: None

This project is discontinued.

Serial No. NDB(I)-67 MN/NR 1415(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Jugularography

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
John L. Doppman

Other Investigators: None

This project is discontinued.

Serial No. NDB(I)-62 MN/AP 926(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electromechanical Coupling in Muscle and Drug Activity

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B. S.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.4 |
| Professional: | 1.4 |
| Other: | 0 |

Project Description:

Objectives: The sequence of physiological events in skeletal muscle which occur between excitation and the development of tension have not been adequately described. No theory of excitation-contraction coupling has been proposed that encompasses all presently known experimental facts. The object of the present study is to establish an ionic basis for the initiation and maintenance of tension in skeletal muscle and to relate the ionic events to electrical and mechanical activity.

Methods Employed: The coupling processes are studied in slow type skeletal muscle fibers. This kind of muscle is used because ionic and electrical events are directly coupled to tension in a graded fashion. Since classical electrophysiological methods have failed to explain fully the coupling processes, ion substitution methods have been used. A new sucrose-gap technique using pairs of isolated muscles and flowing ionic solutions has been developed and operationally used as a convenient means for determining electrical responsiveness at any given point in time. A large variety of quaternary nitrogen compounds have been tested as $Na^+$ substitutes. A group of buffer amines capable of protonation at physiological pH's have also been used successfully as both cationic and anionic substitutes.

Major Findings: The experiments performed during the past year have shown that $Na^+$ currents are involved in the sequence of events which lead to tension development. The evidence is as follows: (1) Isolated slow muscles stay relaxed in solution containing only $Na^+$, $Ca^{++}$, and $Cl^-$. (2) Tension develops in presence of $Na^+$ and $Cl^-$ without $Ca^{++}$. (3) No tension develops with suitable cationic monovalently charged buffers, $Cl^-$ and $Ca^{++}$. (4) No tension occurs in cationic buffer and $Cl^-$ without $Ca^{++}$. (5) During relaxation the replacement of cationic monovalently charged buffer by $Na^+$ without $Ca^{++}$ produces rapid development of tension. (6) During tension the replacement of $Na^+$ by cationic buffers produces relaxation. (7) During tension produced by $Na^+$ return the reentry of $Ca^{++}$ induces relaxation. (8) The substitution of $Cl^-$ by monovalent charged anionic buffers at each step in the substitution experiments fails to influence tension. (9) Physiological amounts of $K^+$ can be present or absent during each step of the substitution experiments without effect on tension. Therefore, of the physiologically present ions, only $Na^+$ can be implicated in the second sequential step which leads to tension development in slow muscle. The first step is the removal of $Ca^{++}$ ions from the most external or superficial phase, the consequence of which is increased $Na^+$ conductance which initiates the next sequential event leading to tension development, possibly by releasing $Ca^{++}$ from phase 2 (sarcotubular reticulum). The released $Ca^{++}$ could then interact with the actomyosin system and initiate ATP splitting and contraction. The experimental findings of this project offer definitive evidence that $Na^+$ current is a sequential step between excitation and contraction. The ongoing experiments treat of further ionic events in the sequence.

Significance to Bio-Medical Research and the Program of the Institute: The research of this project continues to furnish data of a fundamental nature related to muscle function and malfunction. The project is a corollary of the muscle disease research of the Branch.

Proposed Course of Project: The work of the project will continue toward the above stated objective. The currently planned research relates to drug effect and ionic transport systems. Within a few months a marked change in methodology and instrumentation will be required.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-63 MN/AP 1049(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Mechanical Properties of Muscle in Relation to Drug Action

Previous Serial Number: Same

Principal Investigator: Jay B. Wells, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

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|---|---|
| Total: | 0.4 |
| Professional: | 0.4 |
| Other: | 0 |

Project Description:

Objectives: Current investigations of factors that influence contraction would be enhanced if some index or means of muscle classification based on contractile speed were known. Examination of muscle response for such an index was one aim in extending the mechanical characterization of active muscle. A second objective was to complete a reinvestigation of the series elastic properties of slow and fast mammalian twitch muscle using improved apparatus.

Methods Employed: The emphasis of these studies on mechanical responses of muscle has indicated a methodology based on mechanical recording techniques. Tension and displacement transduction systems have been developed with considerable attention toward their properties which in turn increased the fidelity of the muscle measurements and subsequent interpretation.

The data collected for the classification study was based on the active force-velocity relationship of muscle since it is response rate rather than tension or shortening amplitude that distinguishes one muscle from another. All measurements were made on *in situ* mammalian limb muscle preparations.

Major Findings: The following results are based on preliminary evaluation of a small sample from four different muscles in two common species of rodents. Two parameters of the force-velocity relationship, both reflecting the rate of active shortening showed good correlation with the respective rates of isometric tension development. A derivation of the exact relationship between these parameters and the isometric tension rate is expected to produce the desired index which may then be calculated from isometric tension rate measurements.

The results of the reinvestigation of elastic properties confirmed the initial study. The unusual finding was also confirmed that more mechanical energy was recovered during retraction of the series elastic elements than was expected from the experimentally determined stress-strain relation. This result does not support presently accepted models of skeletal muscle. Finally, generalized stress-strain functions for the series elastic elements of the muscles studied have been derived which permit comparison of similar functions from other muscles.

Significance to Bio-Medical Research and the Program of the Institute: The mechanical characteristics of active muscle serve as the standard for evaluating contractile responses of normal or pathologic muscle. This project is designed to expand or clarify these mechanical characteristics in terms of present conceptual models.

Proposed Course of Project: The mathematical description and statistical treatment of data related to the elastic properties of rat limb muscle is essentally completed and the results are currently being prepared for publication.

Expansion of the preliminary results relating force-velocity parameters to some index of contractile speed is indicated by extension of the measurements to other mammalian species. A test of the validity of the index in muscle preparations with unusual or experimentally altered mechanical outputs is also indicated.

Honors and Awards: None

Publications: Wells, Jay B., A relationship between elastic and contractile components in mammalian skeletal muscle. Nature, Lond. 214, 198-199, 1967.

Serial No. NDB(I)-63 MN/AP 1050(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Relationship Between the Functional Activity and the Mechanical Properties of Normal and Modified Striated Muscle

Previous Serial Number: Same

Principal Investigator: Jay B. Wells, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.6 |
| Professional: | 0.6 |
| Other: | 0 |

Project Description:

Objectives: Experiments were designed to determine whether specific changes in the mechanical or neural environments of skeletal muscle changed the mechanical output. Analysis and interpretation of such changes, if any, with respect to trophic or adaptive mechanisms were the main goals of this project.

Methods Employed: Because shortening is a primary property of the joint moving function of limb musculature, its prevention by joint fixation was used to alter the mechanical environment of a slow and fast twitch muscle. Intended modification of neural input was produced in a slow twitch muscle (soleus) by reinnervation with a nerve normally serving the gastrocnemius muscle. The period between joint fixation and mechanical recording was 3 - 5 weeks and the corresponding period for the reinnervation series was 10 - 16 weeks. Sham operated control animals provided the basis for comparison. The data derived from the measurements related to muscle mass, capacity for tension development, contractile speed and some active state properties.

Major Findings: I. Modification of environmental

(mechanical) factors by prevention of shortening produced significant atrophy of both fast and slow muscles as reflected in the 25 - 30% decrease in muscle wet weight compared to that of control. Changes in the capacity for tension development and contractile speed were observed in the responses from the more rapid muscle only. The only positive change observed in the mechanical output of modified slow muscle was a decreased tetanus: twitch tension ratio. A similar change has been observed (elsewhere) during "speeding" of developing fast twitch muscles in rats. II. A changed neural input, by reinnervation of slow twitch muscle with an alien nerve, resulted in muscle hypertrophy as indicated by an increased wet weight of 31% over that of self reinnervated control muscles. However, no change in the capacity for tension development was seen. A significant change in the contractile rate, as reflected in the force-velocity relation constants was also observed. These findings emphasize the changes in mechanical output of muscle which may occur with modification of muscle environment. Such changes are significant clinically as well as from the fundamental aspect of understanding the nature of trophic and adaptive processes in muscle.

Significance to Bio-Medical Research and the Program of the Institute: A mechanical analysis of the naturally occurring or experimentally induced contractile differences observed within the mammalian class is applicable to clinical assessment of muscle function.

Proposed Course of Project: Because muscle output is modified by changes in mechanical and neural environment inquiry into mechanisms involved is indicated.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 MN/AP 1416(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: A Pharmacological and Toxicological Study of Neurotoxic Venoms

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B. S.
Ahmed Hassan Mohamed, Ph.D.
Edward B. Seligmann, Jr., Ph.D.

Cooperating Units: Department of Physiology, Ein Shams University, Cairo, Egypt
Laboratory of Control Activities, DBS, NIH

Man Years:

| | |
|---|---|
| Total: | 0.6 |
| Professional: | 0.6 |
| Other: | 0 |

Project Description:

Objectives: To obtain and investigate heretofore unavailable venomous substances. Attempts are being made to first determine the primary point of biologic activity and then to find out the tissue and cellular mechanisms of neurotoxic activity.

Methods Employed: The above objectives demand the use of a wide variety of whole animal, isolated tissue, and cellular techniques involving toxicological, pharmacological, and biochemical procedures. One or two well-known venoms in each genus are investigated in order to establish a standard. A comparison of the activity of the venoms from the more rare species aids the discovery of new properties of the various venoms with a minimum of search.

Major Findings: The primary effect of each neurotoxic venom studied so far has been a paralytic effect on the neuromuscular system. Complete paralysis is reversible at a small dose but appears irreversible when the dose is large. Cardio-

vascular deterioration occurs later and at greater doses than those which produce paralysis. The changes in electrical activity which occur after venom administration are not observed until the cardiovascular system fails to supply the brain with adequate oxygen. All venoms from the Naja genus can damage the blood brain barrier and change its permeability to large dye molecules. The BBB effect does not occur after intravenous injection at double the $LD_{99}$ dose level but is observed only after intra-arterial administration. The neurotoxin venoms are cytotoxic to many tissues including lung, liver, and kidneys. Hypersensitivity to Naja venoms develops in human beings.

Significance to Bio-Medical Research and the Program of the Institute: Past investigations of venoms have been preeminently successful in various aspects of biology.

The collection of venoms from poisonous species has made possible the scientific development of antivenins for the treatment of envenomation by poisonous animals. The research here is collaborative with and supportive of a program that is now designed to collect venoms from the entire African Continent (except for the Union of South Africa) and to develop polyvalent antivenom for therapeutic use.

Because of the accelerating toxicity to which modern man is exposed, the study of toxic and poisonous substances becomes of increasing importance. Only a few relatively common venoms have been investigated. Over 600 different species of poisonous snakes are believed to exist in the world. What wealth of biological tools are undiscovered in the venom of the lesser known species can only be surmized from the success of past research. The relationships among snake venom neurotoxins, enzymes, antivenins, toxicities, and therapeutics are almost completely unknown. The project, therefore, has excellent possibilities of increasing progress in medical research.

Proposed Course of Project: To continue the research stated above with a broad approach and to ultimately define the more promising areas for investigation so that we may add to our knowledge of the function and dysfunction of nerve and muscle tissue.

Honors and Awards: None

Publications: Irwin, Richard L., and Oliver, Katharine L.: Entry of trypan blue into the rat brain after intra-arterial injection of elapidae venoms. The Pharmacologist, 9: 194, Fall, 1967 .

ANNUAL REPORT
July 1, 1967 through June 30, 1968
Branch of Surgical Neurology, IR
National Institute of Neurological Diseases and Blindness

Maitland Baldwin, M. D., Chief

During the period of this report, this Branch has conducted investigations under the following categorical titles: cerebral edema, cerebral ischemia, cerebral trauma, interhemispheral relationships, language and memory, developmental defects, and epilepsy. Twenty-eight reports were prepared for publication in appropriate journals.

During the period 16 April 1967 through 15 April 1968, 207 persons participated in clinical investigations as inpatients, while 487 were examined as outpatients in a total of 732 visits. There were 125 operative procedures and 103 patients underwent physiological monitoring in the neurosurgical suite.

In the course of these investigations, the Branch has developed active collaboration with the following organizations: Branch of Electroencephalography, NINDB; Laboratory of Neurophysiology, NINDB; Laboratory of Neurochemistry, NINDB; Biometrics Branch, NINDB; Section on Animal Behavior, NIMH; Section of Psychological Research, St. Elizabeth's Hospital; Clinical Center Departments of Clinical Pathology and Diagnostic X-ray; Computer Facilities, DRS, NIH; Biomedical Engineering and Instrumentation Branch, DRS, NIH; Naval Ship Research and Development Center, Department of the Navy.

## Cerebral Edema and Brain Tumor

In the laboratory, further information on the apparent relationship between cerebral permeability and low temperature has been acquired. A pseudohibernator, the Marmota monax, has been subjected to conditions contributory to hibernation in comparison to a control group of peers kept at normal temperatures. These animals were fed silver nitrate ad libidum in the manner described by Wislocki so that hibernating and control groups received regular doses of this compound in their drinking water throughout the experimental periods. When hibernating, the animals proceeded into 'test' drops of 5 to 6 days' duration in which brain temperatures dropped as low as 7° C., and after which they recovered spontaneously so as to eat and drink. The control group ate and drank regularly on a daily schedule. All animals showed stigma of argyria as expected and in accordance with the findings of Wislocki. However, the hibernating group clearly demonstrated deposition of silver salts in the glia of centrum ovale, cortical neurons, pia arachnoid, neurons of the hippocampus and areas other than periependymal gray, area postrema, and hypothalamus, which are usually impregnated during the course of normal thermic argyria. The coincidence of low temperatures consequent to the hibernation state seems relevant to a radical change in cerebral permeability as related to this reliable indicator. This work may be related to that of Brendel, whose biochemical studies suggest that during periods of hibernation which are induced and not spontaneous, a degree of cerebral edema, and therefore cerebral permeability, exists.

In the clinic, two years' experience with intrathecal chemotherapy involving 18 patients with glioblastoma has shown that the first drug tested, Methotrexate, is not a useful agent to use as an intrathecal drug against gliomas; 16 of those 18 patients are dead. The two survivors have been retreated with 8-Azaguanine, the drug currently under test. In one of these patients, a mass which was quite obvious in a previous pneumoencephalogram and is a part of the tumor structure, has vanished, and he is still alive and well three years after his initial cytological diagnosis. As an adjunct to the use of 8-Azaguanine, estimations of desmosterol in the cerebrospinal fluid (Paoletti technique) are being used to monitor the destructive effect of the drug on the tumor.

In a combination of data obtained from studies on cerebral permeability at low temperature, two patients with histologically proven gliomas have been given Vincristine in the amounts of .05 mg./kg. on four successive occasions, correlated with prolonged hypothermia of approximately 24 hours duration. It is estimated that the brain temperatures in these cases ranged around 25° C. at their lowest. These two patients are now alive and well six years after treatment, and are gainfully employed. It is supposed that the correlation of a relatively high blood level of Vincristine with the relatively low brain temperature for periods of more than 30 minutes provides access to the neoplasm by the drug across an otherwise impermeable blood-brain barrier. Recent studies indicate that Vincristine given at normal brain temperatures does not materially affect the biological course of these malignant brain tumors.

Cerebral Ischemia

Resuscitation after acute cerebral ischemia has been the subject of laboratory work during the period of this report. Mongrel dogs are exsanguinated by means of bleeding through the femoral arteries. Ten minutes after exsanguination and when the EKG and EEG records are flat, the animal is rapidly subjected to extracorporeal transcatheter hypothermia, as well as surface cooling of the head. This cooling is continued for at least 20 minutes. During this time, the animal is pumped and oxygenated by intra-arterial catheter as well. Reconstitution of the EKG and EEG for periods ranging from one-half hour (in the original experiment) to 24 hours has been achieved. In order to achieve the necessary perfusion system, a differential cooling technique was devised in which rapid brain cooling to levels between 20° C. and 25° C. is possible through careful placement of the arterial catheter tip in the brachiocephalic arch. These techniques now provide for an operative survival rate of 90 per cent.

Cerebral ischemia is also being studied through re-anastomosis of the severed head of the macaque monkey to another corporeal system. Thus methods have been devised for carotid and jugular re-anastomosis of the hypothermic head of one individual to the hypothermic body of another, while the latter undergoes perfusion oxygenation.

Cerebral Trauma

The natural frequency of brain rotation as measured by radiographic and

lexan calvarium techniques is between 5 to 10 cyles per second (in the monkey).

Our prediction of the injurious effects of a whiplash injury has been confirmed in over 80 monkeys. The levels of rotational velocity and rotational acceleration required for concussion have been defined, as have conditions under which surface brain hemorrhages can be produced by whiplash. Surface hemorrhages are extremely rare as a consequence of direct head injury without skull fracture. The level of rotational velocity at which concussion is produced in 50 per cent of the monkeys is about 50 per cent greater than that required for concussion produced by occipital head impact, a finding which emphasizes the significance of local effects in the production of this syndrome.

Once the rotational frequency of brain and the concussive short-duration rotational velocity thresholds for head impact and whiplash injury are known, the long-duration rotational acceleration thresholds can be calculated and tolerance curves defined in an analogy to a mass-spring system.

A scaling method is being tested in order to extrapolate experimental data for energy thresholds in cerebral concussion of lower primates to similar thresholds in man. This is based on the equation $R + \frac{c}{m^{2/3}}$ where R = rotational acceleration required for injury threshold, m = brain mass in grams, and c = constant derived from our experimental data. At the present time, data are being obtained from the squirrel monkey with a brain weight of 30 gms. and from the chimpanzee with a brain weight of approximately 400 gms.

In the clinic, occult hydrocephalus resultant from severe closed head injury has been studied and partially alleviated by ventriculo-jugular shunting. Seven such patients have been studied in this year. In addition, two well documented cases of post-whiplash hematomas have been observed. One of these was the cause of a fatality in a middle-aged woman three days after the whiplash injury, while the other required operative removal of the clots, which procedure was followed by recovery.

A method for repair of devastating scalp wounds has been devised. The Silvetti technique was modified so as to apply full-thickness calf embryo skin to the denuded calvaria of macaque monkeys. After 12 weeks, the scalp is successfully reconstituted by this method and a normal appearing hair growth covers the replacement.

Interhemispheral Relationships

These have been the subject of study in the laboratory and clinic with the use of surgical, physiological, and psychological techniques. Spectral analysis of electroencephalograms made on chimpanzees after recovery from mesial cerebral incision in which the two hemispheres are separated by this surgical technique shows marked differences. Thus, one or the other hemisphere shows considerably higher power levels than its opposite. Such a chimpanzee is subjected to subtotal hemisection of the brain stem at the infracollicular level so as to sever the afferent and spare the efferents.

There is a marked and further reduction in power levels as recorded from the hemisphere on the side of brain stem section.

A two-week-old chimpanzee was subjected to total mesial cerebral incision and has since been the subject of observational, electrographic, and biochemical study. As of the date of this report, he is five months old. He has a normal nocturnal-diurnal cycle, normal feeding cycle, bilateral synergistic movements, and an EEG characterized by spectral differences as noted above. It is interesting that in some EEG tracings, sleep records can be obtained from one hemisphere, but not from the other, hinting at a duty cycle between the disparate hemispheres. His metabolic studies to date appear normal. In order to test the further effects of the mesial incision in monkeys and chimpanzees, a test pattern has been designed in which the animal is conditioned through either a right-sided or left-sided shock applied to the foot to select from an award-type stimulus located behind doors oriented either on the right, left, or before him. The side of shock stimulus is varied. The animal seems to respond with the simultaneity characteristic of the intact subject.

In a study of the intact callosal function, it has been found that transcallosal volleys are facilitatory in action. Only a small percentage show inhibition of activity of the single cell discharge in response to transcallosal stimulation.

Power spectral analysis of EEG after right or left temporal lobectomy and similar analysis of records taken during and after the intracarotid amytal test are under way. It seems that there may be a correlation between the hemisphere dominant for speech and its power levels as exemplified by the results to date. In any event, it is now clear that intracarotid amytal not only affects the dominant hemisphere with regard to verbal speech, as proposed originally by Wada, but also with regard to recent memory. Moreover, it seems clear that right hemisphere speech is more affected by ipsilateral intracarotid amytal injection than left hemisphere speech.

Language and Memory

Phonemic and dysnomic responses to intracarotid amytal injection and depth electrode stimulation have been the subject of study. Thus the relationship between naming difficulties and the phonemic structure of acquired nouns is being investigated in several types of situations with a small projector and film strips. Disruption in speech has occurred in response to stimulation of left pulvinar, and the left thalamus was shown to be more involved in verbal deficits than the right. In addition, effects of depth stimulation in right pulvinar and right thalamus as displayed during tasks requiring discrimination and short-term memory for non-verbal stimuli, indicate significant memory impairment for non-verbal tasks. The results from this stimulation series suggest that an asymmetry in the organization of the cognitive processes may exist on thalamic level. Thus, left pulvinar may participate in language function and short-term memory, while right pulvinar functions in dealings with non-verbal stimuli. Whereas depth stimulation in left pulvinar produced anomia, left cingulum stimulation in depth

also served to modify naming behavior. In contrast, a greater memory disruption was associated with left cingulum stimulation than with left pulvinar stimulation. No appreciable changes in short-term verbal memory were observed from similar stimulation of right cingulum.

A training plan for brain damaged individuals who suffered dysnomic and amnestic deficits has been developed. In essence, it trains the individual in non-verbal tasks involving spatial, color, and other visual criteria. Then he is trained to communicate, using non-verbal symbols (Morse Code), and finally in mnemonic exercises and speed reading.

In addition to these physiological and psychological studies, serial sections of thalamus following cerebral lesions associated with speech disorders were made in four cases. Near complete degeneration of the pulvinar was seen in a persistent, complete aphasia of three years' duration. Two lesser disorders were associated with pulvinar damage predominantly in the anterior superior portion; in one of these cases it also involved the posterior nuclear dorsalis caudalis. The fourth case, involving a very mild and transient aphasia associated with superficial damage to the supramarginal gyrus, was associated with only a mild cell loss in a small area of the lateral pulvinar. In another unusual case, the pulvinar on the right was nearly completely destroyed, while the left apparently contained viable neuronal structures. This individual retained a comprehensible speech.

Developmental Defects

Further studies on Hunter-Hurler's disease showed that the molecular weights of chondroitin sulfate B and heparitin sulfate which are excessively stored in internal organs and excreted in urines of patients, are considerably lower than in normals. These and other findings indicate that the mucopolysaccharides in this disorder have differing characteristics. Also, the molecular weights of certain mucopolysaccharides were determined precisely for the first time.

Three unusual families with a very high incidence of mental retardation were investigated. Out of a total of 158 subjects in four generations, 69 were retarded. It was concluded that the retardation in these families was primarily the result of genetic factors. The type of inheritance in two families was thought to be dominant, with the possibility of frequent assortative mating being responsible for this dominant trait in a homozygous state in certain parents. In a third family, the inheritance trait was multifactoral.

Eighteen patients in adult age groups with arteriovenous malformations of the spinal cord were studied, using a technique for radiographic demonstration of the arterial supply to these lesions, usually by selective catheterization of the intercostal or lumbar aortic branch from which the vessel arose. Then by a specially designed surgical technique, intradural ligation of the abnormal blood supply was effected. These patients showed dramatic improvement as a result of this combination of new diagnostic and surgical techniques.

## Effects of Ionizing Radiation

Moderately large doses of ionizing radiation (over 250 r) delivered to the human embryo before two to three weeks of gestation are unlikely to produce severe abnormality in children born alive. However, experimental data indicate that considerable numbers of these embryos are resorbed or aborted. Irradiation of the fetus between four and 11 weeks of gestation with doses used in medical therapeutics leads to severe abnormalities, predominantly malformation of many organs of most or all of the children. Radiation in similar dose ranges between 11 and 16 weeks of gestation may produce little or no eye, skeletal, and genital organ abnormalities, but stunted growth, microcephaly, and mental retardation can be expected. Finally, irradiation of the fetus between 16 and 20 weeks of gestation leads to only mild degrees of microcephaly, mental retardation, and stunting of growth, while irradiation after 20 weeks is not likely to produce overt abnormalities leading to handicap in early life.

## Epilepsy

Fifteen children suffering from intractable and frequent epileptic attacks were investigated as inpatients. An effective therapeutic regimen consisting of a combination of anticonvulsants, valium, and corticosteroids on the one hand, or a high ketogenic diet on the other, produced complete control of attacks in these children who had been averaging between 10 and 100 seizures per day.

The effects of amygdaloidectomy, conventional subtotal temporal lobectomy, and other focal excisions, as well as the new combination of hypothermia and intravenous anticonvulsant drugs, have also been studied this year. Stereotaxic amygdaloidectomy has produced gratifying results in reduction of temporal lobe seizures, and particularly in reduction of the concomitant psychiatric aberrations. The results of conventional temporal lobectomy continue to justify the procedure and provide a rich field for electrophysiological studies.

Ten patients suffering intractable seizures have been subjected to general and cranial hypothermia so that the brain temperatures ranged below 25° C. for at least 30 minutes. Then intravenous dilantin in amounts of 4.3 mg./kilo body weight was given over a 10-minute period. As a consequence of this new technique, it has been possible to arrest seizures in otherwise intractable cases in six of these patients over periods ranging from three to six years. In two patients, seizures have been materially reduced so as to permit employment for periods as long as two years. None of these patients were amenable to conventional surgery, and all had long trials on anticonvulsant medication without success.

Serial No. NDB(I)-54 SN/OC 100(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Epileptogenic Mechanisms in the Brain of Man and Other Primates

Previous Serial Number: SAME

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: J. M. Van Buren, M.D., A. K. Ommaya, F.R.C.S., R. Farrier, M.D., C. Ajmone-Marsan, M.D., D. Sadowsky, F. Lamberti, R.N.

Cooperating Units: CC-CP; N-OB; N-EEG; CC-Dir.

Man Years

| | |
|---|---|
| Total: | 2.7 |
| Professional: | 1.5 |
| Other: | 1.2 |

Project Description:

Objectives:

a. To study causal mechanisms of epileptic seizures in man and other primates.
b. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.
c. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.

Methods Employed:

a. Clinical neurological examination.
b. Special radiographic and other contrast examinations.
c. Electrographic, including electrocorticographic, examination.
d. Electrophysiological techniques as indicated.
e. Experimental epileptogenic lesions.
f. Hypothermia.
g. Cerebral drug deposition and scintillation counting of tagged drugs.
h. Histological and chemical examinations as required.

Major Findings: When the epileptogenic brain is subjected to heat exchange so that its average mean temperature is less than 25° C. for a period greater than 30 minutes, the seizure mechanisms within it seem unusually susceptible to intravenous dilantin given in a dose range of 3 mgs./kilo body weight. Thus, if a patient with intractable seizures has not responded to conventional anticonvulsive medication and, because of their nature, the seizures are not amenable to conventional surgical approach, he is subjected to general anesthesia, circumferential head cooling while being physiologically monitored, and otherwise protected against adverse shifts in systemic temperature. He is given dilantin by vein at the optimum time. One such injection has sufficed in most instances. The drug is given slowly over a ten minute period so as not to produce adverse cardiac effects. Recovery from this combination of general anesthesia (inhalation fluothane), cerebral hypothermia, and intravenous dilantin is prompt and relatively free of complications. There are no unusual risks.

To date, 14 patients have undergone this treatment. 10 of these are available for a followup ranging from 7 years to 1 year. Of these ten, eight have had either a radical reduction of their seizures or are seizure free. All were completely incapacitated by the seizure process before treatment, and had been selected for this new therapeutic combination because of the intractable nature of their symptomatology. The age range of these patients is from 28 years to 3 months. Four are female and six are male. Electrographic diagnoses range from centrencephalic epilepsy to somatosensory seizures, and include temporal lobe epilepsy as well. One such patient, a young adult male with psychomotor seizures as well as generalized attacks, has been seizure free for six years after one treatment. His pre-therapeutic course was a dramatic mixture of psychiatric aberration and seizure disorder. He is now gainfully employed. An infant of 3 months has been seizure free for 3 years after a desperate course in status, apparently the result of diffuse cerebral dysrhythmia of subcortical origin. Two young adult patients who owed their temporal lobe attacks to histologically demonstrable gliomas, have been seizure free for 7 and 6 years, respectively, after a combination of intravenous vincristine and hypothermia. They are both gainfully employed.

There are other clues which point to a critical relationship between brain temperature and epileptogenic brain mechanisms. Thus, a young male adult, in status epilepticus because of subcortical seizures projecting to the left temporal and parasylvian region, exhibited a rise of rectal temperature before the onset of his first generalized attack, as well as the predicted and expected slight rise of temperature consequent to the continuing seizures. When his body temperature was reduced so that rectal temperature fell below 37° C. his seizure activity diminished, and as cooling progressed so that his rectal temperature fell below 35° C., seizure activities ceased. When he was rewarmed, either actively or passively, so that rectal temperature returned to its prior levels of 38° C., seizure activity recommenced. It has been known for some time that reduction of cerebral temperature to levels below which the EEG is flat, is concomitant with reduction of epileptogenic activity. It has also been reported that lesser reductions of cerebral temperature of 2 or 3°,

that is to 33° or 34° C., is commensurate with an increase in epileptogenic activity. This has not been the experience of the past year. Moreover, the clinical observation that there is a rise in temperature before the onset of the generalized attack is new and requires further exploitation.

Stereotaxic surgery for epilepsy has been undertaken in this period. Lesions have been made in the amygdaloid nucleus on both sides in cases of temporal epilepsy with marked psychiatric aberrations. On the basis of the short-term followup, the effect on seizures seems to be reduction in frequency and severity, while there are obvious changes in affect. The patients seem more bland and less liable to mood swings, a finding which has been reported recently from Scandinavia, as well as from Germany. On the other hand, lesions of the fields of Forel have not been commensurate with desirable therapeutic effect and will not be pursued for that reason. The conventional subtotal temporal lobectomy continues to yield an effective therapeutic return, as well as the opportunity for neurophysiological exploration of the exposed areas.

Significance to Bio-medical Research and Program of the Institute: These observations have contributed to the knowledge of epilepsy and its treatment.

Proposed Course of Project: An attempt will be made to characterize the biochemical and physiological events surrounding this combination of hypothermia and dilantin. Studies are now underway of CSF levels of dilantin, along with observations of $PO_2$, $CO_2$, arterial and venous pressures. It seems likely that there is a time correlation between the flattening of the EEG, reduction in cerebral oxygen requirements, reduction in cerebral temperature and this drug transfer.

The corpus callosum and its relationship to surgery of epilepsy will be the subject of further investigation based on the experimental developments of mesial cerebral excision on the chimpanzees.

Honors and Awards: None

Publications:

Baldwin, M. and Hofmann,A.: Hallucinations. In Handbook of Clinical Neurology. Amsterdam, North Holland Publ. Co. (In press).

Davie, J. C. and Baldwin, M.: Radiographic-anatomical study of massa intermaedia. J. Neurosurg. 26: 483-487, May 1967.

Serial No. NDB(I)-54 SN/OC 101(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Functional Representation in the Temporal Lobe of Man and Higher Primates

Previous Serial Number: SAME

Principal Investigators: Maitland Baldwin, M.D. and A. K. Ommaya, F.R.C.S.

Other Investigators: J. M. Van Buren, M.D., P. Fedio, Ph.D., H. Lansdell, Ph.D. R. Farrier, M.D., R. Wadeson, M.D., Paul MacLean, M.D., F. Lamberti, R.N., L. Frost, Ph.D.

Cooperating Units: M-LP; N-OB; M-LNP; CC-Dir., PHS-SEH

Man Years:

| | |
|---|---|
| Total: | 2.1 |
| Professional: | 1.1 |
| Other: | 1 |

Project Description:

Objectives: Further understanding of a significant functional representation within the temporal lobe and related structures in man and other primates.

Methods Employed:

a. Electrical stimulation and recording from human, chimpanzee, and monkey temporal lobes; directly, after operative exposure; indirectly by depth electrodes and scalp recordings.
b. Ablation of all or parts of the temporal lobes and limbic systems.
c. Surgical interruptions of connecting pathways.
d. Use of drugs for stimulation and suppression, or other effects, on these systems.
e. Test situation, utilizing machine or automatic programing.
f. Other psychological and physiological examinations.

Major Findings: In order to view the temporal lobe systems in terms of hemisphere function, much of this year's work has been concerned with interhemispheral relationships or with the relationship of temporal cortical physiology to the results of depth stimulation, particularly in thalamus and basal ganglia. In the clinic, patients have been subjected to a diagnostic protocol, the purpose of which is lateralization of speech dominance, but which also provides investigative rewards. Thus, psychophysiological monitoring and

the WADA test demonstrated behavioral differences between patients who showed speech lateralization in the left or right hemisphere. The right hemisphere group was slow and less accurate in the resumption of the name-recall performance test following amytal deactivation on the side of speech. In contrast, the left and right speech groups showed comparable behavioral changes after amytal was injected into the minor or non-dominant speech hemisphere. These findings suggest that when the speech mechanisms are in the right hemisphere there may be reduction in cerebral competence for verbal behavior. Similarly, in a matched series of 10 patients, 5 showed dysnomic defects after amytal injection on the left and 5 on the right. In addition to the dysphasia, a longer lasting impairment of short term verbal memory was also observed; that is, the patients who had recovered motor speech and the naming abilities suffered an appreciable memory impairment following injection into the hemisphere controlling speech. Conversely, injection of the minor or non-dominant hemisphere was associated with only a minimal verbal disruption.

Cortical stimulation results, particularly with reference to sensory responses in mouth, face, head, and neck, were observed during 87 craniotomies for focal epilepsy in 84 patients, and 76 patients with dyskinesias, treated surgically with implanted electrodes. Sensations were reported with stimulation of the pre- and postcentral cortical areas in the convexity of the temporal lobe. Stimulation with depth electrodes produced sensations from loci outside the classical sensory relay areas of the thalamus, from regions in the parietal lobe, and in the vicinity of striatum. The postcentral gyrus showed, as expected, a strongly somatotropic arrangement, with a particularly detailed representation of mouth. Sensory responses from temporal convexity were next most common and showed no somatotropic arrangement. They were usually ipsilateral and showed more frequent representations of head and face. Stimulation of loci in the lateral thalamus and structures antero-superior and postero-superior to the thalamus produced responses which were predominantly contralateral to the stimulus and apt to be referred to combined sites. In view of the differing patterns of sensory representation, it seemed likely that this was based upon anatomically different fiber systems. Although stimulation of these produced subjective sensations, it is suggested that they may serve functions other than simple afferent conduction of peripheral stimuli.

In the Primate Neurology Laboratory, 12 chimpanzees have been subject to mesial cerebral incision and 5 of these animals have also undergone subtotal hemisection of the brain stem at the inferior collicular level after recovery from the mesial cerebral incision. After these procedures, there are no major neurological defects which can be assessed by ordinary observations. Three weeks after the last brain stem section, each subject was immobilized by intravenous succinylcholine and ventilated by intratracheal pump. At that time, a scalp EEG was taken with standard electrode placement, using needle electrodes driven into the outer table of the skull under local anesthesia. Once the preparation was considered stable, it was monitored by pulse rate, blood pressure, skin and rectal temperatures. The EEG and EKG were recorded continuously for one hour. During this time, six channels were recorded on strip charts at a paper speed of 15 mm./sec. Some records were also made on analog

tape. An Ampex FR-1300 portable analog recorder was used for signals obtained from the pre-amplifiers of the Offner with a tape frequency-band width of 1250 Hertz. Strip charts were digitized by means of a Gerber X-Y Scanner, thus obtaining punch card output suitable as input for the IBM 360/50. As many as six traces were digitized simultaneously with a sampling rate of approximately 60 samples per trace per second. The data recorded on analog tape was digitized via a Linc computer which controls the analog to digital conversions and writes a digital magnetic tape suitable as input to the IBM 360/50. Again six channels were digitized simultaneously using sample and hold electronics. A sample rate of 200 samples per channel per second was utilized. Then a spectral analysis was performed. A co-variance function:

$$\Phi_{12}(T) = \lim_{T\to\infty} \frac{1}{2T}\int_{-T}^{T} f_1(t)\, f_2(t+\tau)\, dt$$

was calculated for a pair of EEG signals $f_1$ and $f_2$. A spectral estimate:

$$G(\omega) = \frac{1}{2\pi}\int_{-\infty}^{\infty} \Phi_{12}(T)\, e^{-i\omega t}\, d\tau$$

was then computed.

The preoperative or control record has a broad frequency content as demonstrated by the modulus of the auto-spectra in various traces. After mesial cerebral incision, the EEG records show a marked asymmetry between left and right hemispheres in the frontoparietal regions which is not present in the preoperative tracings. An analysis of the frequency content common to both left and right hemispheres shows that the parieto-occipital regions have a symmetry from .5 to 8 cycles per second (cps.). On the frontoparietal region the amplitudes attributable to common frequencies fall off sharply at 3 cps. After right subtotal hemisection of the brain stem which followed mesial cerebral incision, there was a marked slowing in the EEG. The spectral content of both frontoparietal and parieto-occipital regions is now dominated by low frequency components of less than 4 cps. The cross spectra show that frequencies common to both hemispheres are dominated by components below 4 cps. and decrease sharply above that point.

Significance to Bio-medical Research and Program of the Institute: These findings relate to important cerebral functions such as language, memory and sensation. They further understanding of these complex behavioral substrates, both in health and disease.

Proposed Course of Project: We will continue the studies of inter-hemispheral relationships with particular attention to language and memory functions. In the chimpanzee and the monkey we have established a conditioning system to study simultaneity after mesial cerebral incisions. Similarly, in humans, we have established an audio-input test designed for observation and analysis of simultaneity characteristics in patients with gross hemispheral damage.

Honors and Awards: None

Publications: None

Serial No. NDB(I) - 55 SN/OC 200(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Involuntary Movements

**Previous Serial Number:** Same

**Principal Investigators:** J. M. Van Buren, M. D., Choh-luh Li, M. D., Paul Fedio, Ph.D. and Eugene K. Harris, Ph.D.

**Other Investigators:** H. Weil-Malherbe, M. D. and G. A. Ojemann, M. D.

**Cooperating Units:** Laboratory of Applied Studies, CR; St. Elizabeth's Hospital, NIMH; Department of Neurological Surgery, University of Washington

**Man Years**

| | |
|---|---|
| Total: | 4.0 |
| Professional: | 1.9 |
| Other: | 2.1 |

**Project Description:**

Objectives: Neurological disease characterized by dyskinesias offers a twofold opportunity for research. The pathological aspects of the disease itself may be studied as well as the pathophysiology of the motor system. The second aspect is the unparalleled opportunities afforded for neurophysiological studies in man by stereotaxic surgery in the treatment of dyskinesias. Studies made directly of the disease itself have not received great emphasis in the present research since they require biochemical and pathological support. However, with the collaboration of Dr. Weil-Malherbe at St. Elizabeth's Hospital, new studies in dopamine excretion have been undertaken.

Methods Employed: Surface and depth microelectrode recording of the human brain makes use of specially built high impedance pre-amplifiers, tape recording of signals and eventually averaging and cross correlation of cellular activity and other functions such as muscle activity and brain potential variations from large electrodes. Depth electrode stimulation employs a specially built electrode and a current monitored stimulator. The special apparatus used in quantitative psychological testing is described in Dr. Fedio's Project Report Serial No. NDB(I) - 66 SN/CP 1245(c) and in Dr. Lansdell's and Dr. Fedio's Project Report Serial No. NDB(I) - 63 SN/CP 1033(c). Studies of the catecholamine metabolism and extrapyramidal motor disease is centered largely upon establishing base line normals and the effect of diet upon the urinary excretion of dopamine, both free and conjugated, homovanillic acid and dehydroxyphenylacetic acid.

Major Findings:

(1) Extracellular unit activity in the human thalamus and cortex. As mentioned in last year's report, some thirty tapes have been made. Attempts to analyze this data in terms of unit activity related to sensory inputs with Dr. James Bryan have not been successful despite several revisions of the initial program. Dr. Bryan finally concluded that the present apparatus lacked adequate memory capacity. It also became apparent that an individual was required in our research team who could spend more time and work more closely with the recording personnel. Since the analysis of unit activity also depends upon knowledge of the placement of the electrode and the thalamic variation studies have not been completed [see Project Report Serial No. NDB(I) - 56 SN/OC 304(c)], a final study of this material cannot be undertaken as yet.

It should be pointed out that the analysis of the tapes already available was impeded by the presence of artefact and high noise levels. During the past year when one of us (J. M. Van Buren) who was responsible for obtaining the patients for study was absent, a new amplifier system was designed and built. Efforts to improve the recording of the large depth electrodes are presently in progress under the direction of Dr. Ajmone-Marsan and Dr. Gabor of the EEG Department.

It is hoped with the addition of Dr. Eugene Harris to the team that some of the difficulties arising from lack of adequate computer assistance may be overcome. It would be desirable, however, to have a full-time member of the Department in this capacity [refer to Project Report Serial No. NDB(I) - 62 SN/OC 913(c)].

(2) Depth stimulation.

(a) Sensory responses to depth stimulation. Results of the study of sensory responses elicited from the cerebral cortex and in the depth is reported in Project Report Serial No. NDB(I) - 56 SN/OC 304(c).

(b) Studies of the response to depth stimulation of object-naming tests and tests of form recognition have been carried out.

The anatomic sites associated with disruption in short term verbal memory are the same subcortical areas in which anomic behavior was produced. Stimulation in the left pulvinar was associated with recall disturbance while a moderate degree of impairment was associated with stimulation in the left and right parietal white matter. Stimulation in the right pulvinar and in the left lateral thalamus (outside the pulvinar) did not impair memory performance. No deficit was recorded during stimulation in the left and right temporal white matter (patients with temporal lobe epilepsy).

Further study was directed to evaluate the role of the right thalamus, particularly the pulvinar nucleus, in mediating cognitive behavior. Based upon the nonverbal deficits which frequently accompany lesions in the nondominant hemisphere, a task requiring discrimination and short term memory for nonverbal stimuli (complex designs) was developed. Preliminary observations show that stimulation in the right pulvinar produced significant memory impairment. Moderate degree of recall difficulty was produced by stimulation in the right and left parietal white matter. In each instance, the impairment was observed on the memory aspect of the task; there was no impairment in visual discrimination (matching from sample). The results from the stimulation series suggests that an asymmetry in the organization of cognitive processes appears to exist at the thalamic level. The data demonstrate left pulvinar participation in language functions and short term verbal memory while the right pulvinar subserves mnemonic functions in dealing with nonverbal stimuli or natural which is not readily labeled.

(3) Studies of dopamine excretion in normal subjects and those subject to dyskinesias. The excretion of dopamine (both free and conjugate) and that of the dopamine metabolites homovanillic acid and homoprotocatecholic acid (Dopac) has been studied in a group of patients with extrapyramidal disorders and two groups of control subjects during six consecutive days. Significant differences in excretion rates were found not only between the patients and controls but also between the two control groups; this makes it unlikely that the differences observed were connected with the disease.

The most consistent observation was an effect of the diet on excretion rates. During the first three days the subjects were fed regular meals; during the second period of three days they received a milk preparation (lactum baby food). All excretion rates were appreciably and significantly decreased while the subjects were on the milk diet.

Significance to Bio-medical Research and the Program of the Institute: The use of a human subject who is cooperative and unsedated permits many tests of sensory and psychological function which are impossible even with the time consuming conditioning experiments applied in lower animals. The use of the human subject is therefore not a duplication of animal experimentation but an extension of this and, of course, studies of speech function may only be carried out in man. The effectiveness of the dual physiological-anatomical approach in as difficult an area as speech function as attested by the findings of Ojemann, Fedio and Van Buren recently published (see below) and the confirmatory anatomical studies are reported in Project Report Serial No. NDB(I) - 56 SN/OC 304(c).

Although much has been published regarding catecholamine metabolism in Parkinsonian patients, much of this is very difficult to interpret because of failure to control dietary intake adequately. Dr. Weil-Malherbe's efforts to provide reliable base line studies of catecholamine excretion are thus of considerable significance.

Proposed Course of the Project: Although it has been demonstrated that routine microelectrode recording of extracellular potentials in human subcortical regions is technically feasible, it has been apparent that many technical difficulties must still be worked out. Special attention has been turned to obtaining improved amplifying equipment and to reducing the electrical interference in the 10A operating suite with Mr. Michael Greifner and the cooperation of the Cardiac Surgery Unit. These technical advances and the assistance of Dr. Harris to provide more intensive support in computer processing of data promise to produce more tangible results of studies of depth unit activity in man.

It has been hoped for several years to eventually be able to attack directly the problem of the patho-physiology of motor activity itself. Equipment designed along the lines suggested by Dr. Lawrence Stark, formerly of M.I.T., for relating a visual input to a motor response has been laid aside in the past two years. This has not been due to any lack of interest but simply due to the lack of a person with an adequate engineering and computer background to devote sufficient time to the work in collaboration with the clinical service. The importance of quantitative motor studies for the evaluation of any therapeutic procedures is obvious. Perhaps more important, however, is that the reduction of the complexities of human movement to quantitative terms may permit model making and analysis according to more classical engineering techniques. It seems that a more basic approach to abnormal human movement is important if any advance in the basic understanding of this problem is to be achieved.

Honors and Awards: None

Publications:

Ojemann, G. A., Fedio, P. and Van Buren, J. M.: Anomia from pulvinar and subcortical parietal stimulation. Brain, 91: 99-117, March, 1968.

Serial No. NDB(I) - 56 SN/OC 303(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** The Study of the Functional Anatomy and Pathology of the Human Visual System

**Previous Serial Number:** Same

**Principal Investigator:** J. M. Van Buren, M. D.

**Other Investigators:** Peter Gouras, M. D.

**Cooperating Units:** Ophthalmology Branch, NINDB

**Man Years**
Total: .0
Professional: .0
Other: .0

**Project Description:**

Objectives: The major objectives of this project, which were originally to relate the subjective aspects of vision to anatomy of the human visual pathways, were largely fulfilled in the publishing of the volume, "The Retinal Ganglion Cell Layer." Further material remained, however, for reconstruction studies in the retina. A collaborative study proposed by Dr. Gouras offered the opportunity to assess the stages of retinal degeneration in a dynamic fashion.

Methods Employed: An attempt has been made to add a quantitative aspect to anatomy by reconstruction techniques and these are again proposed for the studies of the retina. Microelectrode recordings from retinal ganglion cells in the intact monkey eye are now feasible without entailing the destruction of the animal after the experiment.

Major Findings: None.

Significance to Bio-medical Research and the Program of the Institute: None.

Proposed Course of the Project: Although the material is available, laboratory interests have turned to other areas. Further work in this area will be reported under the Project Report Serial No. NDB(I) - 56 SN/OC 304(c).

**Honors and Awards:** None.

**Publications:** None.

Serial No. NDB(I) - 56 SN/OC 304(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Effect of Lesions Upon the Function and Structure of the Human Central Nervous System

**Previous Serial Number:** Same

**Principal Investigator:** J. M. Van Buren, M. D.

**Other Investigators:** E. Tarlov, M. D., M. Baldwin, M. D. and P. I. Yakovlev, M. D.

**Cooperating Units:** Warren Anatomical Museum, Harvard Medical School, Boston, Massachusetts

**Man Years**

| | |
|---|---|
| Total: | 1.9 |
| Professional: | 1.4 |
| Other: | 0.5 |

**Project Description:**

Objective: This project is directed toward the study of basic neuroanatomy and neurophysiology in man, making use of pathological material and the opportunities for study afforded by the operative treatment of neurological disease.

Methods Employed:

(1) Anatomical studies:

(a) Serial sections of human and animal brains in celloidin for myelin and Nissl's series.

(b) Section and staining of primate brains with Nauta technique for demonstration of degenerating pathways.

(2) Stimulation of the cerebral cortex during exploratory exposures in the treatment of epilepsy and stimulation deep within the hemisphere during operations for treatment of dyskinesias.

Major Findings:

(1) During the past year the principal investigator undertook nearly full time studies of the thalamic degeneration resulting from human cerebral vascular lesions at the Warren Anatomical Museum. This entailed photographic

reproduction of every 100th section through the hemispheres and every 40th through the thalamus with marking of the areas of degeneration in each series. Following this each of the photographs was traced in ink, the areas of degeneration noted by hatching and the nuclei and major landmarks indicated in letter form. These prints were then photographically reduced and remounted as montages. In this year period a total of 50 cerebra have been completely studied in this fashion with the ink preparation of approximately 2000 drawings. Six cerebra remain to be reconstructed of material currently available at NINDB.

(2) Alterations in speech and the pulvinar. A serial section study of cerebro-thalamic relationships in cases of acquired speech disorders.

Serial section studies of the thalamus following cerebral lesions associated with speech disorders were made in four cases. Near complete degeneration of the pulvinar was seen in a persistent, complete aphasia of three years' duration. Two lesser disorders were associated with pulvinar damage predominantly in the anterior superior portion; in one of these cases it also involved the posterior N. dorsalis caudalis. The fourth case, involving a very mild and transient aphasia associated with superficial damage to the supramarginal gyrus, was associated only with mild cell loss in a small area of the lateral pulvinar.

An unusual paranatal lesion with almost total destruction of both parietal occipital regions and the temporal region on the right and damage to the posterior temporal region on the left permitted the converse observations. In this instance the pulvinar on the right was nearly completely destroyed while on the left apparently viable neuronal structures remained only in the anterior superior and medial portions of the pulvinar. This individual, although functioning at an imbecile level, nevertheless retained comprehensible speech.

In agreement with earlier stimulation studies in the anterior superior pulvinar (Ojemann, G. A., Fedio, P. and Van Buren, J. M., Brain, 91:99-117, 1968), these cases support the importance of the anterior superior pulvinar in speech mechanisms.

(3) Ascending vestibular pathways and the primate brain stem.

This study carried out in macaque, baboon and chimpanzee by Dr. E. Tarlov was designed to test the assertions of Hassler that the vestibular nuclei projected to the V.im.e. of the thalamus. Lesions of the vestibular nuclei were placed in five macaques, one baboon and one chimpanzee and the ascending degeneration studied with the Nauta-Fink method. This showed that degeneration from the vestibular nuclei passed only into the medial longitudinal fasciculus to the oculomotor nuclei and interstitial nuclei. In addition there was extensive degeneration into the dorsal posterior reticular substance. Specifically none of this degeneration entered the dorsal tegmental fasciculus in which Hassler asserted that vestibular efferents passed to

reach the thalamus. In most of the cases, despite attempts to avoid it, there was some degree of damage in the posterior column nuclei and degeneration passed upward in the medial lemniscus to V.c. and also the V.im.e. This inadvertent damage to the posterior column nuclei apparently occurred during the opening of the fourth ventricle to reach the vestibular nuclei.

It thus seems that Hassler's assertions which have received widespread dissemination appear to be false. In the Annual Report of the previous year other brain stem sites distributing to V.im.e. as demonstrated in the chimpanzee were reviewed.

(4) The significance of differing patterns of Mouth, Face, Head-Neck sensory representation in the human cerebrum.

In the cerebral cortex sensory responses from the Precentral, Postcentral and Temporal areas appeared mutually separable with reference to several criteria. The Postcentral stimuli were predominantly Contralateral in character, consisted of Crude Sensation and largely involved a referral to Mouth. Of the Cortical sensory responses one half to two thirds were from the Postcentral gyrus.

Precentral sensory responses were the least common and were characterized by a preponderance of Sensation of Movement and Ipsilateral responses were somewhat more common than Contralateral. Sensations were most commonly referred to Head, followed closely by Mouth referral.

Temporal sensory responses accounted for all the Stato-acoustic Sensations and showed the lowest incidence of Sensation of Movement. The vast majority of these sensations were referred to the Ipsilateral side of the body followed by Bilateral referrals. As opposed to the Postcentral gyrus in which the Mouth representation was predominant, the Temporal region Mouth representation was the least common and referral of sensation to the Head and Neck region was most often seen.

The preponderance of Crude Sensation in the Depth stimulation responses resembled responses of the Postcentral gyrus more closely than either the Precentral or Temporal areas. Likewise the predominantly Contralateral referral in Depth stimulation resembled the Postcentral Cortical referrals. However, in terms of localization of the sensation, Depth stimulation responses most closely resembled those of the Temporal region showing small representation of Mouth and a greater representation of both Face and Head sensation with a striking tendency to be combined with sensations referred to other parts of the body.

Study of the types of sensation disclosed many instances of production not only of Crude paresthesiae but more complicated phenomena resembling the ordinary sensations of daily life. Responses called "Pain" were conspicuous only in their near absence.

The differing patterns of sensory representation found at different areas of stimulation suggests that the responses from these areas are not simply stimulation of the same sensory system at different sites. This evidence and that available in the literature suggests that the ultimate destination of these varying sensory representations is not the cortex itself but rather a subcortical structure and the striatum is strongly suggested. Thus the stimulation sites in the "extra-sensory" regions of the thalamus extending forward into the frontal limb of the internal capsule would represent excitation of fibers en passage of the thalamo-striate system. The first and second somatic sensory receiving areas of the cortex thus represent the true sensory relay cortex and this is supported by the evoked potential studies of Woolsey et al. The Precentral "sensory representation" might well represent a feedback mechanism related to adjusting the motor response. The Temporal Cortical area from which sensory responses were obtained corresponds closely with that pointed out by Penfield as a region from which memories may be elicited by stimulation under exceptional circumstances. This may be more than coincidence and may indicate that this region is important in the storage of sensory information for comparison with later stimuli.

Significance to Bio-medical Research and the Program of the Institute:

(1) The availability of an unusual number of human cerebra with vascular lesions in which the cerebro-thalamic connections could be studied prompted detailed study. A study of this type has never been carried out in man before and indeed, in view of the quantity of material, outstrips any previous study in the lower primates. Since the cerebral damage nearly always involves both cortex and subcortical white matter in man, this poses complications which are usually not seen in the experimental work in lower animals. It is hoped, however, in view of the large amount of material that sufficient overlapping will occur so that this difficulty may, to a degree at least, be overcome.

This study has permitted the principal investigator to become thoroughly familiar with the cytoarchitectonics of the human thalamus and to feel reasonably confident in separating the thalamic subnuclei. This knowledge was essential for study of the variations of the thalamic nuclei with relationship to the ventricular landmarks used in human stereotactic surgery.

(2) To our knowledge the relationship of subcortical structures to speech never has been satisfactorily demonstrated prior to the present studies. The stimulation studies carried out by Ojemann, Fedio and Van Buren, which were carefully controlled to rule out extraneous factors interrupting speech, demonstrated the importance of the anterior superior pulvinar in the dominant hemisphere in object naming. The present cases of aphasia associated with small infarcts in the cerebrum near the commonly accepted speech areas were found incidentally in the thalamic study. It was striking, however, that the areas of degeneration associated with these lesions completely superimposed upon the anterior superior pulvinar regions implicated in speech mechanism in stimulation studies.

(3) The assignment of thalamic functions and systems of function have proceeded at a rapid pace and often on all too thin evidence. The supposition of Hassler's that the vestibular nuclei project to the V.im.e. appears to be an example of this and detailed studies have given no support to this hypothesis. More detailed studies of thalamic connections particularly in the high primates and man are needed if many unfounded assumptions are not to mislead us in clinical work.

(4) The multiple "sensory" representations have been difficult to interpret over the years and the finding in man of a further "sensory" representation in the temporal lobe has compounded the difficulty. The present hypothesis does away with this problem to a degree by suggesting, firstly, that the cortex is not the ultimate destination of sensory impulses. In addition the multiple areas in the cerebrum from which sensory responses may be elicited, in addition to being direct afferent pathways, may represent reentrant pathways used in motor control and in the establishment of sensory storage areas used for comparison with subsequent sensory input.

Proposed Course of Project:

(1) The major effort of the principal investigator is to finish the studies of the human diencephalon.

It is now planned to publish two volumes, the first being the cytoarchitectonic definition of the thalamic nuclei and their cerebral connections, and the second volume being a variation atlas of the human diencephalon with particular attention to the variations of the thalamic nuclei. Since these variations will be taken with respect to the radiologically demonstrable third ventricular landmarks, the second volume will provide a guidebook for the stereotactic surgeon and for those wishing a reasonable anatomical control of depth electrode placements and lesions.

(2) In support of this work further studies will be undertaken in higher primates to define the brain stem afferents and their regions of projection in the thalamus.

Honors and Awards: None

Publications:

Van Buren, J. M.: Studies in human hypophyseal ablative procedures. Arch. Otolaryng. 86: 268-273, 1967.

Van Buren, J. M., Ommaya, A. K. and Ketcham, A. S.: Ten years experience with radical combined craniofacial resection of malignant tumors of the paranasal sinuses. J. Neurosurg. 28: 341-350, 1968.

Serial No. NDB(I)-61 SN/OC 802(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Single Cell Discharges in Motor Cortex in Response to Stimulation of Basal Ganglia and Cerebellum

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

This project is to be discontinued. A new research program related to this has been initiated with the collaboration of the Laboratory of Neurophysiology, NIMH and the Laboratory of Applied Studies, DCRT.

Serial No. NDB(I)-61 SN/OC 803(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cortical Intracellular Potentials

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

This project was carried out in order to meet the assignment extended by the Czechoslovakia Academy of Science. Since the assignment has been met and the results of this study published, this project was terminated.

Serial No. NDB(I) - 62 SN/OC 906(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** The Effects of Cold and the Relation of Temperature to Functions of the Central Nervous System

**Previous Serial Number:** Same

**Principal Investigator:** A. K. Ommaya, F.R.C.S., F.A.C.S.

**Other Investigators:** None

**Cooperating Units:** None

**Man Years**

| | |
|---|---|
| Total: | .3 |
| Professional: | .3 |
| Other: | .0 |

**Project Description:**

Objective: A study of comparative temperature gradients in nervous and other tissues under various normal and abnormal conditions and in response to heating and cooling of small areas (in cats).

Methods Employed: The following instrumentation and techniques are being used or developed:

1. A 24-point multiple-pattern thermocouple array for temperature gradient recording. This array consists of micro-thermocouples arranged around a central probe shaft which can be used for heating, cooling, or biopsy. The data are to be recorded through a Dymec data acquisition system on punched tape, thus facilitating analysis.
2. A differential temperature thermocouple to study related problems in situations where the 24-probe array cannot be used.
3. Angiographic x-ray demonstration of zones of freezing block and other changes in blood vessels of cats.
4. Construction of a new system for focal heating and cooling within the nervous system.
5. A series of temperature gradient measurements are being made in water, gelatin and other gels, and fluids of known viscoelastic properties.
6. The development of a thermal neurosurgical probe incorporating a resistance heater at the tip of a 1 mm diameter probe carrying six thermocouple junctions along the probe length. This enables the investigation of reversible heat inhibition of neuronal functions as well as the creation of heat lesions within brain tissue.

Major Findings: Because work on this project has been temporarily discontinued no major findings can be reported as yet. The thermal probe has been built and continuing work is restricted to perfecting the read-out system for the six thermocouples and current input to the resistance heater tip.

Significance to Biomedical Research and the Program of the Institute: The direct applications of the findings on freezing lesions have been described previously and published. The temperature gradient study will provide basic data on the comparative thermal properties of brain and other tissue which will allow direct estimation of thermodynamic aspects of their function. From points of difference and/or similarity, considerable insight could be obtained into the nature of these functions in vivo.

Proposed Course: The analysis of normal and abnormal temperature gradients and thermal properties of tissues will continue.

Honors and Awards: None

Publications: None

Serial No. NDB(I) - 62 SN/OC 907(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Trauma to the Nervous System. Experimental and Clinical.

**Previous Serial Number:** Same

**Principal Investigator:** A. K. Ommaya, F.R.C.S., F.A.C.S.

**Other Investigators:** A.E. Hirsch, Ph.D., J. Geschwin, M.S., R.M. Mahone, M.S., P. Corrao, M.D., D. Sass, M.D., D.B. Gainsburg, M.D., K. Parker, B.S., H. Metz, B.S., R. Bourke, M.D. S. Tocek, M.D., W.H. Marshall, M.D., J.H. Hubbard, M.D.

**Cooperating Units:** Personnel Protection Branch, Naval Ship Research and Development Center; Laboratory of Biophysics, NMRI; Laboratory of Neurochemistry, NINDB; Georgetown University Neurosurgical Service, D. C. General Hospital; Laboratory of Neurophysiology, NIMH.

**Man Years**

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 1.0 |
| Other: | 1.0 |

**Project Description:**

Objective: A clinical and experimental study of the biophysics, physiology and pathology of trauma and its effects on the central nervous system in order to enable prediction of the results of such trauma in man and to establish its management on a rational basis.

Methods Employed:

1. The Field Emission type of ultra-high-speed x-ray apparatus is now in use and preliminary experiments with carotid angiography and radiopaque markers are in progress.
2. Lexan calvaria are being prepared and studied by high-speed cinematography during impact for measuring actual brain displacements.
3. The electrical impedance, EEG, and D.C. potential changes of the monkey cortex are being recorded under supervision of and in the laboratory of Dr. Wade Marshall. The response of these observations to various concentrations of $CO_2$ before and after experimental concussion is being pursued in collaboration with Dr. Marshall.

4. The arterial and C.S.F., $pO_2$, $pCO_2$, and pH are being measured simultaneously in patients after severe head injuries and in monkeys before, during and after experimental concussion.

5. An apparatus able to provide controlled degrees of impulsive loading ("whiplash") to the animal without direct head impact has been constructed and is now in use to supplement information previously obtained from our head impact device.

6. A hyperbaric chamber is being fitted with a "concussion gun" to enable the study of experimental head injury and its effect under controlled conditions of atmospheric composition and pressure.

7. A technique for chronic implantation and recording from cortical and deeper brain structures via fine stainless steel and platinum electrodes is being developed. Four monkeys have been prepared with a total of 8 bipolar depth electrodes and 6 unipolar surface electrodes in each animal. Electrophysiological studies (slow wave phenomena as well as evoked potentials and extracellular "neuronal pools") will be recorded before and after experimental head injury.

8. Modified Florey-Barr brain observation chambers have been developed. These will allow the administration of precise degrees of focal cortical trauma while allowing simultaneous visual (cinematographic) observations as well as measurement of gas tensions in the C.S.F. overlying the damaged area and concurrent recording and of EEG and D.C. potentials.

9. Isotope cisternography and ventriculography are being used to diagnose occult hydrocephalus, a late sequela of severe head injuries. The relationship between this complication and post-traumatic epilepsy is also being investigated in selected patients.

10. The silicone rubber cerebrospinal fluid reservoir is being used to record intraventricular pressures over long periods of time in selected patients with severe brain trauma.

11. In collaboration with Drs. Bourke and Tocek, a small group of head injury patients will be intensively studied and the data coded according to the system developed. The primary intention is the development of a logical classification of head injuries.

Major Findings:

1. The natural frequency of brain rotation as measured by the radiographic and lexan calvarium techniques is between 5 to 10 cycles per second (in the monkey).

2. Our prediction of the injurious effects of a whiplash injury has been confirmed in over 80 monkeys. In addition to defining the levels of rotational velocity and rotational acceleration required for concussion, we have demonstrated the regular production of surface brain hemorrhages (mainly subdural) by whiplash injury. It should be emphasized that surface hemorrhages are extremely rarely obtained by direct head impact injury in monkeys (short of skull fracture). The level of rotational velocity at which concussion is produced in 50% of monkeys is about 50% greater than that required for concussion produced by occipital head impact indicating the significance of local effects.

3. Knowing the rotational frequency of brain as well as the concussive short-duration rotational velocity thresholds for head impact and whiplash injury we can calculate the long-duration rotational acceleration thresholds and define tolerance curves based on the analogy to a mass-spring system.

4. Our scaling method dependent on an inverse 2/3 relationship of brain mass is being tested to extrapolate experimental data for energy thresholds for cerebral concussion in lower primates to thresholds for cerebral concussion in man. This is based on the equation $R = \frac{c}{m^{2/3}}$ where R = rotational acceleration required for injury threshold; m = brain mass in grams and c = a constant derived from our experimental data. At the present time we are obtaining experimental data for the squirrel monkey (brain wt. = 30 gm), and for the chimpanzee (brain wt. = 400 gm). Extrapolation to the human brain weight will enable us to obtain an indication of the injury threshold for concussion in man (in terms of angular head velocity and acceleration). Final confirmation of this prediction will be sought from a careful analysis of accident data in man.

5. In studies on C.S.F. $O_2$ tensions, we have determined the dynamic relationship between $pO_2$ in this fluid with relation to the $pO_2$ of arterial blood in the monkey. We have also shown a species difference in the response of cortical tissue $pO_2$ to hyperoxygenation. Thus in monkeys, increased serum $pO_2$ is associated with a fall in cortical $pO_2$, while in rats a rise in brain $pO_2$ is seen. The implications of these observations for brain vascular autoregulatory activities in the more highly evolved species are worthy of further investigation.

6. Occult hydrocephalus is a fairly common occurrence after severe closed head injury in man and alleviation by ventriculo-jugular shunting can result in significant clinical improvement as late as three years after apparent stasis in the neurological/intellectual deficits. A total of seven patients has been studied in the past year.

7. Two well documented cases of post-whiplash subclinical hematomas have come to our attention. One of these was fatal in a middle-aged woman as early as three days after the whiplash injury and the other necessitated surgical removal of the clots. These clinical experiences and our experimental results have enabled us to advise physicians to treat severe whiplash injuries not only from the musculoskeletal and cervical nerve root aspects but also with the realization that cerebral concussion and other sequelae of a closed head injury may have occurred.

8. Aspects of this research have been reported in publications listed under Project Serial No. NDB(I) - 63 MN/NR 1047(c) and Serial No. M - NC - 35.

Significance to Biomedical Research and the Program of the Institute:

1. The ability to define an injury threshold for cerebral concussion and other effects of nervous system trauma in man suggests the possibility of a more adequate definition of safety standards for protection against head injury and whiplash injury in automobile accidents and motorcycle crashes.

2. The findings that a cervical collar raises the threshold for experimental cerebral concussion in the monkey suggest a number of ways in which protection against such injury may be achieved under a variety of conditions. Currently an inflatable collar device and an inertia strap for helmet restraint are being tested.

3. The findings that whiplash injury alone can produce significant degrees of visible brain surface hemorrhages raise the issue of such lesions being produced by whiplash trauma in man (as commonly occurs after rear-end automobile collisions). Further work is required to confirm these observations.

Proposed Course:

1. Rheological studies will be made on fresh in vitro and in vivo samples of nervous tissues to establish fundamental data on the viscoelastic properties of these tissues and their behavior under stress.

2. Input from the above and from the ongoing mechanical, biochemical, and physiological studies will be used to develop the theoretical analysis and produce a theoretical model for the behavior of the brain in response to trauma.

3. Controlled "whiplash" and whole-body acceleration trauma experiments in monkeys will be conducted to round out other aspects of the mechanics of injury.

4. Prophylactic and therapeutic measures aimed at reducing the noxious and enhancing the beneficial responses after trauma will be studied experimentally in the monkey.

5. Detailed neuropathological studies are now being conducted with Dr. P. Corrao in the laboratory of Dr. Snodgrass at the Naval Medical Research Institute.

6. Experimental verification of our scaling method ($R = \frac{c}{m^{2/3}}$) using the squirrel monkey and chimpanzee as well as the rhesus will then allow testing of the prediction of concussive threshold for man against available accident data.

7. An investigation of cerebral blood flow and C.S.F. dynamics, memory and other behavioral disturbances in head injured patients will be conducted in collaboration with Georgetown University. This will be combined with the testing of a method of coding and classifying head injury patients admitted to the Accident Service of the Georgetown University Hospital.

Honors and Awards: None

Publications:

Ommaya, A.K., Yarnell, P., Hirsch, A.E., and Harris, E.H.: Scaling of experimental data on cerebral concussion in sub-human primates to concussion threshold for man. Proceedings of the 11th Stapp Car Crash Conference. New York, Soc. of Automotive Engineers, pp. 47-52, Oct. 1967.

Yarnell, P., Charlton, G., Merril, C.R., Ghiardi, F., and Ommaya, A.K.: Dynamics of oxygen tension in the cisternal cerebrospinal fluid of the rhesus monkey. J. Neurosurg. 27: 515-524, Dec. 1967.

Faas, F., and Ommaya, A.K.: Brain tissue electrolytes and water content in experimental concussion in the monkey. J. Neurosurg. 28: 137-144, Feb. 1968.

Ommaya, A.K.: Mechanical properties of tissues of the nervous system. J. Biomechanics 2: 1-12, April 1968.

Ommaya, A.K., Yarnell, P., and Faas, F.: Whiplash injury and brain damage: An experimental study. J.A.M.A. 204: 285-289, April 1968.

Ommaya, A.K., Di Chiro, G., Baldwin, M., and Pennybacker, J.B.: Non-traumatic cerebrospinal fluid rhinorrhea. J. Neurol. Neurosurg. Psychiat. (In press).

Ommaya, A.K., Boretos, J.W., and Beile, E.E.: The lexan calvarium: An improved method for direct observation of the brain. J. Neurosurg. (In press).

Hirsch, A.E., Ommaya, A.K., and Mahone, R.H.: Tolerance of the sub-human primate brain to cerebral concussion. In Conference Proceedings of the Impact Injury and Crash Protection Symposium, May 9-10, 1968, Wayne State University (In press).

Serial No. NDB(I)-62 SN/OC 913(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Single Cell Discharges from Various Nervous Structures and Their Functional Organization in Particular Reference to Somatosensory Activity in Man

Previous Serial Number: SAME

Principal Investigators: Choh-luh Li, M.D., Ph.D., John Van Buren, M.D., Ph.D.

Other Investigators: Eugene Harris, Ph.D., Anthony F. Bak, Raymond Mejia, B.S.

Cooperating Units: CR-LAS; M-LNP; CR-DSA

Man Years:

| | |
|---|---|
| Total: | 2.8 |
| Professional: | 1.5 |
| Other: | 1.3 |

Project Description:

Objectives:

a. To extend the experimental observations from laboratory studies to clinical surgical procedures in order to gain further understanding of motor function in man.
b. In addition, responses of cells in various brain structures to visual, tactile, and auditory stimulation are investigated.

Methods Employed:

a. Multiple electrodes are placed in the motor cortex, thalamus, caudate nucleus and pulvinar.
b. Extracellular action potentials from these structures are simultaneously recorded before, during, and after, the voluntary and involuntary movements of patients subject to operations.
c. Responses to tactile, visual, and auditory stimulation are also recorded. This datum is stored in magnetic tapes and is to be converted into digital signals, ready for computer analysis.

Major Findings: At the time of this report the investigators are still

involved in technological refinement, and no conclusive statement can be made.

Significance to Bio-medical Research and Program of the Institute: This study will add to our understanding of motor function or dysfunction in man and eventually provide benefits to patients with motor disorders.

Proposed Course of Project: This is a continuation of the project titled: Single Cell Discharges from Motor Cortex and Basal Structures of Man. It will be continued further and eventually extended to the study of the cerebellum.

Honors and Awards: None

Publications: None

Serial No. NDB(I) - 63 SN/OC 1025(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Tumors of the Nervous System

**Previous Serial Number:** Same

**Principal Investigator:** A. K. Ommaya, F.R.C.S., F.A.C.S.

**Other Investigators:** P. Paoletti, M.D., R. Paoletti, M.D.

**Cooperating Units:** Neurosurgical Clinic and Institute of Pharmacology, University of Milan, Italy

**Man Years**

| | |
|---|---|
| Total: | .8 |
| Professional: | .4 |
| Other: | .4 |

**Project Description:**

Objective: To understand how gliomas develop and grow and how best they can be treated in man.

Methods Employed:

1. A special silicone device described as a cerebrospinal fluid reservoir has been designed by the principal investigator. This allows sterile access to the ventricular cerebrospinal fluid by subcutaneous puncture. This device allows utilization of the cerebrospinal fluid pathways for introduction of cytotoxic agents for chemotherapy, either by infusion or perfusion.

2. Estimation of desmosterol in the cerebrospinal fluid has been shown by the Paolettis to be a dependable way of indicating the presence of gliomas, particularly after preliminary blockage of cholesterol synthesis with Triparanol in patients with this type of brain tumors only. Arrangements have been made to freeze samples of cerebrospinal fluid from all patients admitted to this project. These frozen samples will be sent for estimations of desmosterol to the Paolettis in Milan with the object of testing three hypotheses:

a. that desmosterol levels in the cerebrospinal fluid accurately indicate the presence of gliomas;
b. that higher levels of desmosterol will occur at times of accelerated growth of the tumor and that these times will be used to determine timing of therapy;

c. that the established desmosterol levels will accurately reflect the destructive effect of 8-Azaguanine on glioblastoma multiforme and will thus serve as a suitable index for dosage and duration of chemotherapy.

Major Findings: Because of the inadequate collaboration that extended from the National Cancer Institute as detailed in last year's report, it was decided to terminate that arrangement and continue this project as an entirely Surgical Neurology Branch/NINDB one. Extreme scarcity of beds remains a problem and currently this has been palliated by the use of two beds from the National Institute of Mental Health. This arrangement is the result of exploring possible collaborative efforts on the biogenic amines in the cerebrospinal fluid with Dr. I. Kopin and on the relationship between cerebrospinal fluid pressures and sleep mechanisms with Dr. Snyder in such patients. These arrangements will be reported as new projects in the next year.

A review of the two years of intrathecal chemotherapy involving eighteen patients with glioblastoma has shown that the first drug tested, Methotrexate, is not a useful agent to use as an intrathecal drug against gliomas; sixteen of those eighteen patients are dead. The two survivors have been retreated with 8-Azaguanine, the current drug under test. In one patient a previously obvious mass seen on pneumoencephalography has vanished and he is alive and well three years after the initial diagnosis.

Aspects of this research have been reported in a publication listed under Project Serial No. NDB(I) - 56 SN/OC 304(c).

Significance to Biomedical Research and the Program of the Institute: It is hoped to improve the presently inadequate treatment of glial tumors of the nervous system, and hopefully extend clinical cures to patients with glioblastoma multiforme and other malignant gliomas.

Proposed Course: To continue investigation of patients with glioblastoma along the lines indicated in order to determine the validity of the hypotheses outlined above.

Honors and Awards: None

Publications:

Bering, E.A., Jr., Rall, D.P., Walker, M., Leventhal, C., and Ommaya, A.K.: Intrathecal chemotherapy of gliomas: Rationale and current status. In Proceedings of the Conference on Research in the Experimental and Clinical Aspects of Brain Tumors, Feb. 26-27, 1968, Bulletin New York Academy of Sciences (In press).

Serial No. NDB(I)-65 SN/OC 1205(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neurosurgical Monitoring

Previous Serial Number: SAME

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: Eugene Harris, Ph.D., Robert Farrier, M.D., A. K. Ommaya, F.R.C.S., Choh-luh Li, M.D., J. M. Van Buren, M.D., Paul Fedio, Ph.D., M. Haddox, R.N., Raymond Mejia, B.S.

Cooperating Units: R-BEI; CR-DSA

Man Years

| | |
|---|---|
| Total: | 4.5 |
| Professional: | 2 |
| Other: | 2.5 |

Project Description:

Objectives:

1. Physiological parameters of neurosurgical operations.
2. Processing of data thus derived.
3. Estimation of clinical and physiological significance of correlations obtained from such data.
4. Physiological effects of cortical and subcortical electrical stimulation.

Methods Employed:

1. Application of the Sanborn Physiologic Data Monitoring System.
2. Recording from patients during operation.
3. Recording from non-operated patients.
4. Recording from normal controls.
5. Data programming by the Computer Branch in accordance with a collaborative plan.

Major Findings: During the period of this report, 103 patients underwent physiological monitoring in the Neurosurgical Suite. This procedure combines physiological monitoring with psychological testing, EEG, and depth stimulation. The results of these procedures are reported in the following relevant projects: 100(c), 101(c), 1245(c) and 1424(c).

Significance to Bio-medical Research and Program of the Institute: This equipment and the program utilize the wide opportunity for clinical observation of patients undergoing complex neurosurgical procedures, and also provide physiological data useful in the study of the human nervous system.

Proposed Course of Project: Since the development of the facility for this extensive monitoring is now complete and the results from its use will be reported under appropriate research projects, the reporting of Neurosurgical Monitoring, as an entity, is being discontinued.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 SN/OC 1206(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Microbial Characteristics in a Neurosurgical Environment

Previous Serial Number: SAME

Principal Investigator: Maitland Baldwin, M.D.

Other Investigators: L. G. Herman, Ph.D., D. G. Fox, Ph.D., R. J. Weatherby, F. Lamberti, R.N., Delta Trickett, R.N.

Cooperating Units: R-ES; CC-EHS

Man Years

| | |
|---|---|
| Total: | 2 |
| Professional: | 1 |
| Other: | 1 |

Project Description:

Objectives: Analysis of salient microbial features in a neurosurgical room environment.

Methods Employed:

a. Personnel and environmental microbial sampling.
b. Analysis of physical environment.
c. Analysis of personnel structure.
d. Analysis of laminar flow system and comparison with conventional system.

Major Findings: This year's work was concerned with systematic and periodic surveillance of the neurosurgical environment. The results of this surveillance is still under programming and not available for investigative conclusion at the time of this report. The developmental contribution from this project during this report period was a change in scrub technique for surgical procedures. As a result of microbial surveillance of skin surfaces, before, during and after scrub procedures, it was determined that the traditional brush and detergent method was no longer necessary. A far simpler method using plastic sponges impregnated with hexachlorophene is now in use and skin sampling from the systematic procedures indicates that the results of this one-step "soft scrub" are at least as effective as the traditional, prolonged abrasive methods.

Significance to Bio-medical Research and Program of the Institute: This project contributes to further development and modernization of surgical operating techniques, particularly in relation to operative exposure of the nervous system.

Proposed Course of the Project: The results of systematic microbiological surveillance will be used for conclusions relating to changes in various environmental conditions, particularly ventilation, draping and lighting.

Honors and Awards: None

Publications:

Baldwin, M. and Fox, D. G.: Laminar flow for the Neurosurgical Operating Room. J. Neurosurg. (In press).

Serial No. NDB(I)-67 SN/OC 1417(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Changes in Physiological Properties of Brain Tissue at Low Temperatures and in Other Pathological Conditions

Previous Serial Number: SAME

Principal Investigators: Choh-luh Li, M.D., Ph.D., Igor Klatzo, M.D., Joseph Fenstermacher, Ph.D.

Other Investigators: Barbro Johansson, M.D., Anthony F. Bak

Cooperating Units: C-LCP; N-LNNS; M-LNP

Man Years:

| | |
|---|---|
| Total: | .2 |
| Professional: | .2 |
| Other: | 0 |

Project Description:

Objective: To investigate changes in electrophysiological properties and histological manifestations of the brain tissue under various conditions.

Methods Employed:

a. Cats under Fluothane anesthesia are used.
b. Some of the animals are subject to hypothermia and the others serve as controls.
c. Action potentials and resting potentials are recorded from single nerve cells in the cerebral cortex, specific resistivity of the cortex and white matter is determined, spontaneous electrical activity and transcallosal evoked responses are registered. These data are simultaneously obtained at a given temperature, which is continuously measured from the cortex and white matter with thermisters; they are stored in magnetic tapes or photographic film to be analyzed by either a Linc computer or manual measurements.
d. The extracellular volume at temperatures of 36°C and 15°C is determined by the concentration profile of radio-active inulin in the periventricular gray matter. The radio-active inulin is perfused into the lateral ventricle through a small cannula and is recovered from the posterior fossa. The radio-active

perfusate introduced and collected, as well as any possible amount of radio-active substance in the general blood circulation, is also quantitatively determined.

e. Experimental brain edema is produced by injection of liquid paraffin or peanut oil into the external carotid artery. In some animals it is also produced by injection of Triethyltin.
f. Experimental hypertension is produced and the change in the blood pressure and electrical activity of the cortex is continuously recorded by a polygraph.
g. The brain is removed at the end of the experiment and is examined with various histological techniques and, in some cases, with electronmicroscopy.

Major Findings:

a. In normothermic animals the extracellular volume of the gray matter of the brain is 13.5% of the total volume, when it is determined by the electrophysiological method, and is 13.8% by the inulin concentration profile method. At low temperatures the study has not been computed, and a conclusive statement cannot be made at this time; although the electronmicroscopic study appears to indicate that there is no change in the extracellular space.
b. In hypothermic animals the resting potentials of the cortical neurons are unchanged. However, in the process of cooling the frequency of single nerve cell discharges shows an increase and decrease and finally complete disappearance at 20°C. This phenomenon also occurs in the spontaneous electrical activity and evoked potentials recorded from the cortical surface. In hypothermic animals there is an inhibition of passive transport of radio-active inulin and sucrose from the ventricle to the extracellular compartment.
c. Electrophysiological measurements conclusively differentiate extracellular from intracellular edema and prove to be valuable in the collaborative investigation of histopathological tissue of the brain.

Significance to Bio-medical Research and Program of the Institute: The electrophysiological study indicates that there is no swelling of nerve cells in the brain, even when the brain temperature is lowered to 12°C. This gives assurance to our patients, who are subject to hypothermia, if the hypothermia procedure is properly carried out.

It is of considerable importance that at normal body temperature the volume of the extracellular compartment is found to be consistant, as it is determined by two entirely different methods. The value thus obtained is slightly larger than those reported by neurochemists. In all cases, however, skepticism has been raised by biophysicists. The present study serves as a stimulation for further and more accurate determination of extracellular

space which is believed to be of importance in the understanding of brain metabolism.

Proposed Course of Project: It is planned that a collaborative effort will be made with Dr. Kenneth S. Cole to determine the extracellular volume of brain tissue.

Honors and Awards: None

Publications:

Li, C-L., Ohta, T., Ojemann, G., and Parker, L.: Some physiological observations on cats under hypothermia by carotid-carotid perfusion. J. Exp. Neurol. (In press) 1968

Serial No. NDB(I) - 67 SN/OC 1418(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Surgical Treatment of Arteriovenous Aneurysms (Malformations) of the Spinal Cord

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, F.R.C.S., F.A.C.S.

Other Investigators: G. Di Chiro, M.D., J. Doppman, M.D.

Cooperating Units: Section of Neuroradiology, Medical Neurology, NINDB; Diagnostic X-ray Department, Clinical Center, NIH

Man Years

| | |
|---|---|
| Total: | .6 |
| Professional: | .3 |
| Other: | .3 |

Project Description:

Objective: To establish the pathological anatomy and natural history of spinal arteriovenous malformations in order to provide the best surgical treatment for such lesions in man.

Methods Employed: Selective aortographic techniques for radiologic demonstration of the arterial blood supply of such lesions have been developed by Drs. John Doppman and Giovanni Di Chiro (see Individual Project Reports by these investigators on diagnostic aspects of this project) based on the experience of Djinjian, Faure and their co-workers in Paris, France. These workers had applied the technique of extradural and often extraspinal ligation of arterial blood supply of such lesions. We have investigated the effect of intradural ligation of such vessels "feeding" the malformation in eleven patients.

Major Findings: Eighteen patients with this disease have been studied to date. Once the arterial supply is clearly demonstrated, usually by the selective catheterization of the intercostal or lumbar aortic branch from which the vessel arises, the surgical technique for exposure and intradural ligation of the abnormal blood supply is now well established by our experience. Extremely dramatic improvement in neurological deficits has occurred in at least 6 patients, the others improving either slightly or showing arrest in an otherwise progressive neurological deficit. Not a single patient has

been made worse by this procedure. This compares very favorably with any other form of treatment and offers hope of permanent relief since a follow-up of nearly three years is now available for our first three patients. It has also been noted that the shorter the duration of symptoms and neurological deficit, the more rapid and complete is the dissipation of such deficit after this type of surgery.

Aspects of this research have been reported in publications listed under Project Serial No. NDB(I) - 65 MN/NR 1195(c) and CC-26.

Significance to Biomedical Research and the Program of the Institute: In addition to providing a solution to this clinical problem, our surgical method and diagnostic studies provide an opportunity to contribute significantly to the knowledge of the normal and abnormal blood supply of the human spinal cord as well as to related aspects of spinal cord physiology and pathology.

Proposed Course: To obtain a large series of such patients and follow them for a significantly long period of time in order to answer the intentions of the objectives stated for this project.

Honors and Awards: Certificate of Merit at the Scientific Exhibit of the 68th Annual Meeting American Roentgenray Society, September 26-29, 1967

Publications:

See Major Findings above.

Serial No. NDB(I) - 68 SN/OC 1522(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Neural Control of Visuo-Motor Learning and Memory

**Previous Serial Number:** None

**Principal Investigator:** A. K. Ommaya, F.R.C.S., F.A.C.S.

**Other Investigators:** J. Bossom, Ph.D., P. Fedio, Ph.D.

**Cooperating Units:** Section on Clinical Psychology, SN/NINDB; Primate Laboratory, Brooklyn College, New York

**Man Years**

| | |
|---|---|
| Total: | .6 |
| Professional: | .3 |
| Other: | .3 |

**Project Description:**

Objectives:

1. To analyze the neural basis of prism adaptation as a tool to study visuo-motor mechanisms.
2. To determine the mutual roles of complexity and time in the mechanism of memory and develop a predictive theory of memory.
3. To ascertain the role of the limbic system in memory and visuo-motor learning.

Methods Employed:

1. Prism adaptation in monkeys combined with classical lesion analysis using behavioral techniques is the main technique. A test for reaching-grasping behavior has been developed by Dr. Joseph Bossom.
2. A matching from sample memory test capable of controlled variation of time and complexity of task has been developed.
3. Selected patients admitted for surgical relief of either pain or epilepsy often need depth electrodes implanted in either the cingulum or amygdala. Psychologic and memory tests before and after stimulation and coagulative electrode lesions at these sites provide important data for analysis. Stimulation of these structures at various parameters of current and frequency also are proving very rewarding.

Major Findings:

1. Bilateral dorsal rhizotomy in monkeys (C2 to T4) does not obliterate reaching-grasping and prism adaptation is unimpaired. This finding supported older theories of a "motor corrollary discharge" capable of providing sufficient "local feed-back" in the brain to perform useful motor acts. Lesions in the temporal, striate and parietal cortex do not impair this mechanism which is inhibited only by frontal lobe lesions (involving head of caudate nucleus or bilateral stereotaxic caudate nucleus lesions).

2. The matching from sample memory test has been well tested in a group of 20 normal volunteers and preliminary scoring established.

3. Cingulum stimulation impairs short-term memory much more when it is in the dominant hemisphere and also qualitatively more decisively than equivalent stimulation of the amygdala (right or left).

4. Unilateral stereotaxic amygdalotomy has produced significant improvement in behavior and seizures in a small group of patients.

Significance to Biomedical Research and the Program of the Institute: It is hoped that the understanding of visuo-motor learning and memory will lead to a more accurate system of guiding the acquisition of neonatal and infant motor skills as well as improve the rationale and quality of rehabilitation after severe neurologic disease with crippling sequelae.

Proposed Course: To continue on the three aspects of the project, one of which is in collaboration with Dr. Bossom with whom the bulk of the behavioral testing in monkeys is carried out at the Brooklyn College, New York.

Honors and Awards: None

Publications:

Bossom, J., and Ommaya, A.K.: Visuo-motor adaptation (to prismatic transformation of the retinal image) in monkeys with bilateral dorsal rhizotomy. Brain 91: 161-172, March 1968.

Serial No. NDB(I)-68 SN/OC 1524(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electrical Resistivity of Brain Tissues

Previous Serial Number: NONE

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Anthony F. Bak

Cooperating Unit: M-LNP

Man Years:

| | |
|---|---|
| Total: | .5 |
| Professional: | .5 |
| Other: | 0 |

Project Description:

Objective: To determine the specific electrical resistivity of the brain tissue and impedance change in single nerve cell membranes.

Methods Employed:

a. Cats under Nembutal anesthesia are used.
b. A small cranial opening is made, and the temperature of the cortex and its underlying white matter is continuously monitored by thermistors.
c. A pool is built around the bony opening and is filled with an electrolyte solution having the same osmolarity and electrical resistance as the cortex. The selection of this solution is a result of a series of experiments.
d. Four microelectrodes are mounted on the same plane, and the distance between the tips is measured under a microscope.
e. The electrodes are lowered in small steps from the solution in the pool to the cortex and white matter.
f. Constant current pulses are delivered to the peripheral electrodes, and IR drops are recorded from the central electrodes.
g. Micropipette electrodes inserted into the interior of the neuron are used for the study of impedance change in single nerve cells.
h. Constant current pulses are delivered through the micropipette, one depolarizing and two hyperpolarizing. The two hyperpolarizing

pulses are identical, in strength and duration, and are applied one before and one after the depolarizing pulse.

i. The amplitudes of the hyperpolarizing pulses recorded by the micropipette electrode are taken as a measure of the impedance change of the cell membrane.

Major Findings:

a. At a temperature of 36° C the specific resistivity of the cerebral cortex is found to be 556 ± 45 (S.D.) and that of the white matter 580 ± 53 (S.D.) ohm cm.
b. The limiting membrane of the electrically inexcitable cells in the cortex has a much lower resistance than that of the excitable neurons.
c. Immediately following excitation for a period of 50-100 milliseconds, or longer, there is a decrease in the resistance of the membrane of the neuron.

Significance to Bio-medical Research and Program of the Institute: The findings of the present study are not in agreement with reports from other laboratories. Since electrical resistance changes are known to occur with various physiological states of the brain and specific resistance of the brain tissue has been used for estimating the extracellular volume of the cortex and white matter, the results of this study are pertinent in the research in this field.

Proposed Course of Project: With improvement and modification of technology, specific resistivity of the cell membrane of different types of cells under different physiological conditions will be determined.

Honors and Awards: None

Publications:

Li, C-L., Bak, A.F., and Parker, L.O.: Specific resistivity of the cerebral cortex and white matter. J. Exp. Neurol. March, 1968.

Serial No. NDB(I)-68 SN/OC 1525(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electrical Resistivity of Biophysical Models

Previous Serial Number: NONE

Principal Investigators: Kenneth S. Cole, Ph.D., Choh-luh Li, M.D., Ph.D., Anthony F. Bak

Other Investigators: NONE

Cooperating Units: N-LB; M-LNP

Man Years:
Total: .3
Professional: .3
Other: 0

Project Description:

Objective: To evaluate the applicability of the Maxwell Equation for estimating cellular volumes in suspensions consisting of artificial cells of different sizes and shapes in electrolyzed solutions.

Methods Employed:

a. Two electrodes are placed in a specially constructed container. The electrodes are made of stainless steel plates, triangular in shape, with a contact surface of approximately 18 sq. in. and a thickness of 0.002 in.
b. Between the two electrodes are suspensions consisting of different sized and shaped artificial cells in electrolyte solutions of various concentrations.
c. Alternating current is passed from one electrode to the other at frequencies of 0.1, 1.0 and 10.0 kilocycles per second.
d. The specific resistivity of the suspension between the two electrodes is measured by means of an impedance comparer. The temperature of the electrolyte solution of the suspension is measured.

Major Findings: Since this research project was initiated toward the end of 1967, a great deal of discussion and experimentation has been made in regard to the material and the technique employed. At this stage of

investigation no conclusive statement about the observations can be made. However, it is apparent that this model can be used for the evaluation of the Maxwell Equation and its application to the study of extracellular space of the nervous tissues under different experimental conditions.

Significance to Bio-medical Research and Program of the Institute: In the past eight years extensive study has been made on the subject of impedance changes of the brain tissues in animals of different physiological states. These changes are believed to be related to the change of the size of nerve cells and the change in extracellular space of the nervous tissue in the brain. However, the basic mechanism underlying this change in electrical measurements is not completely understood. The present investigation is designed for further understanding of this mechanism. The results of this study will serve as a test for the mathematical equation derived by one of the principal investigators (Cole) approximately 30 years ago.

Proposed Course of Project: This study will be extended from biophysical models to nervous tissues for further evaluation of the validity of the results reported by various investigators.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 SN/OC 1526(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Epileptic Neurons and Their Recurrent Axon Collaterals

Previous Serial Number: NONE

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Vazha Okujava, M.D. (Visiting scientist from U.S.S.R.), Anthony F. Bak

Cooperating Units: N-EEG; M-LNP

Man Years:
Total: .5
Professional: .5
Other: 0

Project Description:

Objective: To study the electrical epileptic nerve cells in the cerebral cortex.

Methods Employed:

a. Cats under ether and d-tubocurarine or under pentobarbitone sodium are used.
b. A glass micropipette electrode is positioned in the interior of a nerve cell in the cerebral cortex.
c. Electrical current of various intensity and duration is applied through the micropipette, and the electrical activity of the cell is recorded.
d. Stimuli are also applied to the surface of the cortex adjacent to the microelectrode. Surface electrical activity is also recorded.

Major Findings:

a. Depolarization of the nerve cell membrane with a constant current of appropriate strength and duration causes the cell to discharge at high frequencies comparable to epileptic firing. The termination of depolarization sets off a prolonged hyperpolarization. There is no afterdischarge of the cell. This

is in contrast to the observation usually obtained in epileptic cells produced by other methods.

b. Repetitive depolarization with short duration, constant current pulses at frequencies of 20-50 per second, evokes repetitive discharges of the cell. The termination of the repetitive depolarization again gives rise to a prolonged hyperpolarization during which time small ripples of inhibitory postsynaptic potentials appear. Again, there is no afterdischarge.

c. In both instances (a and b) there is no change in activity recorded from the cortical surface 1 mm. from the cell under study.

d. Hyperpolarization of the cell abolishes the activity of the cell with absence of miniature excitatory postsynaptic potentials.

e. Anodal break responses may occur, depending upon the level of the resting membrane potential and the state of the discharge activity of the cell, before the application of the hyperpolarizing current. In all instances there is no afterdischarge and no change in the surface cortical activity.

f. Repetitive stimulation applied to the cortical surface causes a gradual depolarization of the cell membrane with high frequency discharges. Epileptic afterdischarges appear on the surface associated with bursts of cell discharges.

Significance to Bio-medical Research and Program of the Institute: This study provides conclusive evidence that there is a negative feed-back system; and the recipient neuron for the impinging impulses, which are generated by the neuron and travel along the recurrent axon collaterals, is the neuron itself. This is accounted for by the small ripples of potential and prolonged hyperpolarization following the termination of the repetitive depolarization pulses. The afterdischarges and epileptic activity require massive and synchronous synaptic activation which can occur only in a large population of cells. However, the size of a population capable of producing epilepsy in a clinical sense remains to be determined.

Proposed Course of Project: The present investigation leads to the understanding of the mechanism of epilepsy and much more effort will be directed to this goal.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 SN/OC 1527(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Physiological Mechanism of Motor Function in the Cat

Previous Serial Number: NONE

Principal Investigators: Choh-luh Li, M.D., Ph.D., Eugene Harris, Ph.D., Anthony F. Bak, Raymond Mejia, B.S.

Other Investigators: None

Cooperating Units: CR-LAS; M-LNP; CR-DSA

Man Years:

| | |
|---|---|
| Total: | .2 |
| Professional: | .2 |
| Other: | 0 |

Project Description:

Objective: To study the functional organization of the pyramidal and extrapyramidal pathway.

Methods Employed:

a. Multiple extracellular microelectrodes are placed in the motor cortex, nucleus ventralis lateralis of the thalamus, caudate nucleus, putamen, globus pallidus, red nucleus and dentate nuclei of the cerebellum.
b. Spontaneous extracellular spike action potentials are simultaneously recorded and stored in magnetic tapes.
c. The spike action potentials are converted into digital signals, and their relationship is to be computer analyzed.
d. Stimulations of various frequencies and strengths are applied to the dentate nucleus and cerebral peduncle. The responses of the cells in various structures are simultaneously recorded on tape and analyzed by the computer.
e. In some animals the motor cortex or one of the subcortical nuclear structures had been chronically destroyed and the relationship of the single cell discharges before and during stimulation is, likewise, analyzed.

Major Findings: The preliminary observation reveals that the dentate

nucleus of the cerebellum exerts strong influence on the activity of cells in the ipsilateral and contralateral red nucleus and of the somatosensory cortex. On some occasions spontaneous reciprocal activity of the cells in the motor cortex on the ipsilateral and contralateral side was seen. On other occasions the cell discharge in the red nucleus was facilitated by stimulation of the ipsilateral dentate and inhibited by the contralateral dentate, or vice versa. Finally, stimulation of the red nucleus on one side was found to evoke discharges from cells in the red nucleus on the opposite side. The results of other experiments will be further analyzed by the computer.

Significance to Bio-medical Research and Program of the Institute: Much has been learned about the functional connections at the cellular level between the cerebellar nuclei, basal ganglia and motor cortex (J. Neurosurg. Supp. Jan. 1966, Part II: 222-226 J. Exp. Neurol. 14: 319-327, March, 1966). The results of these studies were obtained with one or two extracellular microelectrodes simultaneously recording from one or two cells in the pyramidal and extrapyramidal pathways. With the present improved technology (Bak) and knowledge of mathematics and computer (Harris), it is anticipated that with four or six microelectrodes recording simultaneously from different nuclear structures, a great deal more could be learned from the experiments presently designed.

Proposed Course of Project: This study will cover all the structures in the central nervous system related to motor function and will eventually be extended to study the functional relationship between the motor and sensory system.

Honors and Awards: None

Publications: None

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Experimental Hypothermia

Previous Serial Number: SAME

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: Y. Kondo, M.D., A. K. Ommaya, F.R.C.S., R. Farrier, M.D., Choh-luh Li, M.D., F. Lamberti, R.N.

Cooperating Units: R-BEI; CC-Dir.

Man Years
Total: 4.6
Professional: 3
Other: 1.6

Project Description:

Objectives:

a. To study the effects of low temperature on the brain.
b. To study various related physiological parameters.
c. The development of topical and selective cooling techniques potentially available for clinical application.

Methods Employed: The methods employed are body surface cooling, topical cooling of the exposed brain, and/or spinal cord, intravascular perfusion, and combinations of these.

Major Findings: A previous finding indicative of a change in blood-brain barrier when brain temperature reaches levels at or below 25° C., as well as evidence indicating protection from effects of cerebral ischemia during profound hypothermia, led to further studies designed to provide a safe means for development and maintenance of satisfactory cerebral temperatures. Thus, profound differential cooling of the brain has been the object of this year's investigation. A technique for the differential cooling of the brain previously reported by means of pump oxygenator (J. Neurosurg. 24: 1966) was employed, and superficial head cooling was combined in some cases. 59 mongrel dogs were used for this study. Rapid brain cooling to levels approximating 20° C. was easily obtained by means of an extracorporeal perfusion in which the tip of an arterio-catheter was placed at the branching of the main arteries passing into the intercranial spaces. The survival rate of chronic preparations was 90%. However, cardiac temperatures were difficult to control and often remained in hazardous ranges. When superficial head cooling was combined with perfusion cooling and drainage of blood from the right atrium

into the pump oxygenator, brain temperature was easily maintained at about 20° C., while heart temperatures stayed above 28° C. and always remained within safe ranges.

Significance to Bio-medical Research and Program of the Institute: The aims of this project have been and are the provision of safer methods of clinical hypothermia usable as protection against cerebral ischemia and in combination with drug therapy.

Proposed Course of Project: The techniques devised will be used extensively in the further development of an experimental model for the study of acute cerebral ischemia, such as produced during cardiac arrest or stroke.

Honors and Awards: None

Publications:

Baldwin, M.: Cerebral argyria after hibernation. Neurology (In press).

Ohta, T. and Baldwin, M.: Mechanical stimulation at the Circle of Willis. J. Neurosurg. (In press).

Serial No. NDB(I)-60 SN/CN 706(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Clinical, Biochemical and Genetical Studies of Mental Retardation, Progressive Cerebral Degeneration and Cerebral Palsy in Children

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: George Constantopoulos, Ph.D. and Joseph H. Zelson, M.D.

Cooperative Units: None

Man Years

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 1.4 |
| Other: | 0.6 |

Project Description:

Objectives: Actually this is a large permanent program. Chronic neurological disorders affecting children constitute one of the major medical and social problems of our times. According to conservative estimates for this country, about four million children are permanently handicapped by mental retardation, cerebral palsy, epilepsy or progressive cerebral degeneration. Many of them have to be cared for to the end of their life by the joined services of the society and government. Until recently these conditions did not receive sufficient attention in the field of medical research. Our main objectives are:

1. To subdivide the large number of studied patients with mental retardation, cerebral palsy and epilepsy into distinct clinical categories using developmental and neurological examinations along with special laboratory procedures.

2. To establish types of cerebral lesion characteristic of each category whenever possible.

3. To apply basic biochemical and genetic methods to selected diseases or syndromes within each category with the aim of advancing the underlying pathogenesis and etiology.

4. To advance when possible the therapy and prevention of these chronic diseases.

Methods Employed:

1. Detailed general medical and neurological studies.
2. Developmental and psychological examinations.
3. Modified electroencephalographic studies.
4. Biochemical assays of protein, lipid and carbohydrate metabolism.
5. Genetic and cytogenetic studies.
6. Employment of special procedures such as pneumoencephalography and biochemical studies of brain biopsies whenever these are performed for diagnostic reasons.

Patient Material: 32 inpatients and 44 outpatients including consultations.

Clinical Project

Major Findings:

1. Thirty-two inpatients and 44 outpatients were studied and treated during the last year. Fifteen out of 32 inpatients were epileptic children with intractable seizures of usually cryptogenic type; eight had mental retardation of familial type; seven had inborn error of metabolism affecting the brain and two suffered of congenital malformation or cerebral palsy. The children studied as outpatients presented various types of epilepsy, cerebral palsy, mental retardation and progressive cerebral degeneration.

2. Three unusual families with a very high incidence of mental retardation were investigated in great detail. Out of a total of 158 subjects in 4 generations, 69 were retarded. The appraisal was very complete and included social competence, scholastic and psychological evaluations in addition to general, neurological and developmental examinations. Four to six members from each family were hospitalized for the purpose of longer observation and special laboratory studies which included assays of protein, carbohydrate and lipid metabolism, EEG, pneumoencephalography and endocrinological studies.

These families presented several unique features from the genetic viewpoint: (a) A great number of illegitimate children by different men were born to several of the retarded females; (b) A proportion of siblings was reared in good foster homes since birth thereby providing a degree of internal control. On the basis of overall analysis it was concluded that the retardation in these families was a result of genetic factors primarily, although the environment might have contributed slightly. The type of inheritance in two families was thought to be dominant with a possibility that frequent assortative mating was responsible for the dominant trait to be in a homozygous state in certain parents. The type of inheritance in the third family was thought to be multifactorial, since there was a marked variability in the degree of retardation. Clearly, in situations like this some type of eugenic planning is indicated.

3. Our invesitgations of the patients with Hunter-Hurler's syndrome brought about several significant findings which are being currently prepared for publications. We have improved the methods of isolation, separation and identification of acid mucopolysaccharides (AMPS) which permitted quantitative determination, whereas previously up to 45 percent of the material was lost during processing. This permitted a more realistic characterization of individual AMPS such as heparitin sulfate and chondroitin sulfate B including their molecular weights.

4. We have demonstrated that lymphocytes of Hunter-Hurler's patients have specific AMPS inclusions in their cytoplasms and that the ratio of lymphocytes with inclusions is constant for the particular patient and his affected relatives. The ratio permits to distinguish between at least two variants of the disease. The biology (protein production, survival rates) of the inclusion containing lymphocytes was studied in considerable detail and a report is being prepared for publication.

5. Other studies concern production and modification of the production of AMPS by the patients and by the fibroblasts in tissue culture derived from these patients. The fibroblasts perpetuate the metabolic error. The accumulation of these data has to continue before final evaluation is made.

Significance to Bio-Medical Research and the Program of the Institute: The described findings under 1-5 constitute significant contribution to the research on metabolic and chronic neurological disorders in children.

Proposed Course of the Project: This field of study is one of the most important in medicine at the present time; therefore, this program is expected to continue for many years to come.

Honors and Awards:

1. Clinical Assistant Professor of Neurology, George Washington University.
2. Consultant, District of Columbia Children's Hospital.

Publications:

Dekaban, A. S. and Klein, D.: Familial mental retardation. Acta. Genet. 18: 206-228, 1968.

Serial No. NDB(I)-60 SN/CN 707(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Study of Pathological Lesions of the Central Nervous System Occurring During Prenatal, Intranatal and Early Postnatal Life

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.3 |
| Other: | 0.4 |

Project Description:

Objectives: In the majority of patients the causation and the underlying pathology of mental deficiency and cerebral palsy are poorly understood. Detailed histological, histochemical and biochemical examination of the brains of such children are conducted. The main purpose is to correlate the encountered lesions with the clinical data. The accumulation of a larger body of data is expected to provide valuable information regarding pathogenesis and also clues for certain etiological factors. Limited, experimental approach in animals is used whenever indicated.

Methods Employed:

1. Detailed evaluation of the clinical and laboratory data of children who suffer from chronic neurological disorders.

2. Gross examination of brains after their demise.

3. Microscopical study of large histological sections stained by a variety of chromatic, myelin and silver methods as well as by special histochemical procedures.

4. Histochemical and chemical studies of brains and organs of patients who died of progressive cerebral degeneration and of inborn errors of metabolism affecting the nervous system.

5. Tissue culture of affected organs of patients with metabolic diseases with a view of studying cellular metabolism.

Material: Brains of patients who died with a diagnosis of cerebral palsy, epilepsy, mental deficiency or progressive cerebral degeneration. Brains of experimental animals.

Major Findings: Clinical-pathological correlations in certain neurological disorders are still scanty and there exists a need for clues to point the direction toward better directed therapeutic and preventive measures.

1. During the past year we have performed six autopsies on patients who suffered from cerebral palsy, mental retardation and progressive cerebral degeneration. Their brains were processed through large histological section, studied histochemically and described. The accumulation of this valuable material has to continue before statistical analysis can be attempted.

2. Severe and obvious abnormalities encountered in 26 children who received heavy x-irradiation during various stages of gestation were compiled and evaluated. The following conditions occurred most frequently: stunted growth, microcephaly, mental retardation, microphthalmus, pigmentary degeneration of the retina, cataracts, genital and skeletal anomalies. A tentative timetable for man is presented which correlates specific types of abnormalities with irradiation during particular stages of gestation when the dose is in the range of therapeutic radiation. On the basis of the presently available patient material and with some support of experimental data, the following generalizations are made: (a) Moderately large dose of ionizing radiation (over 250r but the upper limit cannot be stated) delivered to the human embryo before 2-3 weeks of gestation is not very likely to produce severe abnormality in most of the children born, although experimental data indicate that considerable numbers of these embryos would be resorbed or aborted; (b) Irradiation of the fetus with doses used in medical therapeutics between 4 and 11 weeks of gestation would lead to severe abnormalities (predominantly malformation) of many organs in most or all of the children; (c) Radiation in similar dose range between 11 and 16 weeks of gestation may produce little or no eye (late cataracts not considered), skeletal and genital organ abnormalities but stunted growth, microcephaly and mental retardation would be frequently present; (d) Irradiation of the fetus between 16 and 20 weeks of gestation with similar dose range may lead only to mild degrees of microcephaly, mental retardation and stunting of growth; (e) Irradiation of the fetus with similar dose range after 20 weeks of gestation is not likely to produce overt abnormalities leading to a serious handicap in early life. However, a proportion of the infants may show evidence of radiation exposure such as skin erythema, abnormal pigmentation, epilation, or deficiencies in the hematopoietic system.

Significance to Bio-Medical Research and the Program of the Institute: Knowledge of detailed pathology and pathogenesis of numerous conditions which underlie cerebral palsy, mental retardation and progressive cerebral degeneration is of basic importance for eventual therapeutic and preventive measures. Some of our studies in this field are of applied type and some as

the above quoted have basic implication of vulnerability and selective ability at regeneration of the developing brain. The disorders listed are clearly the major responsibility of our institute.

Proposed Course of the Project: This project has a wide scope and pertains to one of our major interests. It supplements also our principal clinical project, NINDB-74(c); therefore, it is our permanent project.

Honors and Awards: None

Publications:

Dekaban, A. S.: Abnormalities in children exposed to x-radiation during various stages of gestation - tentative timetable of radiation injury to the human fetus. J. Nuclear Med. (In press).

Serial No. NDB(I)-60 SN/CN 708(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cytogenetical Study of Human Chromosomes Especially in Patients With Congenital Malformations and Mongoloids and Their Families

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigator: Joseph H. Zelson, M.D.

Man Years

| | |
|---|---|
| Total: | 0.8 |
| Professional: | 0.5 |
| Other: | 0.3 |

Project Description:

Objectives: A proportion of patients with congenital malformations and mental retardation have chromosomal aberrations. The aberrations may occur sporadically, in which case they represent an accident of meiosis or division during early cleavage or the chromosomal aberrations may be present in many members of the family. In this last instance it is heritable according to the laws of Mendel.

Methods Employed:

1. Comprehensive clinical, laboratory and pedigree investigation of selected patients to establish diagnosis and extent of abnormality.

2. Tissue culture of lymphocytes from the peripheral blood and fibroblasts from skin. Bacto-Phytohemagglutinin is added in order to stimulate lymphocyte proliferation. Six hours prior to sacrificing the culture colchicine is added in a final concentration of $0.5 \times 10^{-6}$. Fibroblast cultures are similarly processed.

3. Through a number of steps the white blood cell suspension is centrifuged and washed, then exposed to hypotonic salt solution and fixed.

4. Concentrated suspension of cells is stained on the slide with orcein, Giemsa or Feulgen methods.

5. Under high power resolution in light and contrast phase microscope the chromosomes are counted, their morphology studied and karyotype figures made for final analysis.

6. In selected patients, DNA duplication sequence is followed in the chromosomes during the late phase of lymphocyte culture using tritium labelled thymidine.

Material: Patients with congenital malformations, mental retardation and mongolism, as well as their families, are the subject of this study. An experimental approach on human tissue cultures is also used.

Major Findings:

1. Examination of chromosomes was carried out in 15 patients and in 10 persons from their immediate families. Also, chromosome service was provided to the investigators in other institutes. Out of 15 patients tested, 8 showed chromosomal abnormalities in a form of trisomy, monosomy, translocation or morphological aberrations. These findings provided important diagnostic information for a proportion of our patients and helped in formulating family counseling.

2. One of the very important and resistant tasks in human genetics is mapping of the chromosomes. Among many patients whom we studied and on whom we performed karyotypes, we have encountered a unique situation: a child with symptoms, signs and laboratory data of incomplete mongolism had also a partial trisomy G. The extra chromosome is only two-thirds in length of the smallest chromosomes. Autoradiography and special measurements suggest that this extra chromosome has a deleted portion and is trisomic for Group G chromosomes. Thus, lack of frequent characteristics of mongolism such as brachycephaly, palmar dermatoglyphics, typical pelvic index and others may indicate that these traits are located on the distal portion of the long arm which is deleted in the 47th chromosome of trisomy G in this patient. This finding will increase information for mapping 21-22 chromosomes in man.

Significance to Bio-Medical Research and the Program of the Institute: Study of abnormal chromosomes in clinical syndromes provides the only mean whereby human chromosomes could be mapped; the importance of this cannot be overemphasized. The etiology and pathogenesis of a vast number of conditions associated with congenital malformation and mental deficiency are largely unknown. Demonstration of chromosomal aberration in some of these patients or their parents is a great step forward in our understanding of these conditions and it may suggest certain preventive measures.

Proposed Course of the Project: Because of the smallness of staff and space allotted to our section, this project has to be greatly scaled down. We will continue to study somatic chromosome abnormalities in our patients, but experimental approach and study of meiotic chromosomes cannot be carried out.

Honors and Awards: None

Publications:

Dekaban, A. S. and Zelson, J.: Mental retardation in a child with 47 chromosomes - the extra element being submetacentric and shorter than 21-22 group; question of partial mongolism. J. Ment. Defic. Res. (In press).

Serial No. NDB(I)-63 SN/CN 1026(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Clinical and Biochemical Studies in Epilepsy

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigator: Joseph H. Zelson, M.D.

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.8 |
| Professional: | 0.5 |
| Other: | 0.3 |

Project Description:

Objectives: Idiopathic epilepsy is a clinical syndrome of unknown etiologies. In the first instance it is important to accumulate a sufficient number of patients for the purpose of subdividing them into different categories depending on certain clinical and laboratory findings. This will permit better directed therapeutic application of certain regimens and drugs under investigation. The patient's response or its lack may provide us with some informations on the pathogenesis of the condition.

Methods Employed:

1. Necessary clinical examinations and tests.
2. Routine and special electroencephalographic studies.
3. Pneumoencephalography and arteriography when indicated.
4. Urinary and plasma amino acids.
5. Assays of carbohydrate metabolism.
6. Plasma lipid studies.
7. Endocrinological assays when indicated.
8. Application of special therapeutic procedures.
9. Special studies on high fat diet.

Patient Material: 15 inpatients and 9 outpatients, most of them suffering from "idiopathic" epilepsy.

Clinical Project

Major Findings:

1. Fifteen children suffering from intractable and frequent epileptic attacks (10-100 minor spells per day) were investigated as inpatients. Nine of these were developing normally prior to the onset of seizures; in five the initial development was slightly slow and one child was retarded since birth but had no motor deficits. Using intensive treatment, either in a form of combined antiepileptic drugs, valium and corticosteroids, or if these all failed administering the high fat diet, the attacks were completely controlled in eight and the remainder showed a significant decrease in frequency. We have observed again that concomitant with cessation or reduction in seizures, the overall performance improved and the development accelerated.

2. The children established and maintained on the high fat diet provided an opportunity to study the associated biochemical changes dependent on this rigorous dietary regimen. Important observations were reported in Arch. Neurol. 15: 177-189, 1966, and others are currently evaluated.

3. Special diet was devised for experimental animals to parallel our clinical study of children on high fat diet. The biochemical and physiological responses are currently under investigation.

Significance to Bio-Medical Research and the Program of the Institute: Epilepsy is a frequent disorder in all age groups and especially during childhood. Our understanding of the pathogenesis of seizures and their control is still meager. Many young children became severely and permanently retarded as a result of frequent seizures. Biochemical approach to epilepsy is likely to provide us with better understanding of this syndrome complex and it may help us in the therapy of this distressing disorder. The investigation enlarges the overall scope of the study of epilepsy which is a major theme of the Surgical Neurology Branch, NINDB.

Proposed Course of the Project: The project will continue for years to come.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 SN/CN 1523(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Lipid and Polysaccharide Composition of the White and Grey Matter of the Brain

Previous Serial Number: None

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigator: George Constantopoulos, Ph.D.

Cooperative Units: None

Man Years

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.3 |
| Others: | 0.4 |

Project Description:

Objectives: Many of our patients with progressive cerebral degenerations have either excessive storage of a material in the nervous system (lipid, mucopolysaccharide, glycogen) or loss of structural material (diffuse sclerosis, subacute sclerosing encephalitis, axonal degeneration). Initially we plan to use experimental animals to establish techniques of analysis of biochemical partitioned structural components as outlined above. In later phases human material, both normal and abnormal, will be utilized as it becomes available.

Methods Employed:

1. Rapid removal of the brain and its preparation for histological, histochemical and biochemical studies. Histological methods will include staining for myelin, nerve fibers and perikaria as well as histochemical techniques for enzymes.

2. Biochemical investigations will be conducted on submicroscopic fractions of the brain. Defined portions of the brain will be taken for lipid, mucopolysaccharide and protein studies.

3. Experimental animals will be injected with labelled isotopes to permit study of incorporation of certain substrates.

Material: 1. Rats - adult and young.
2. Human cerebral tissue as it becomes available.

Major Findings: This project is in its initial stage.

1. Determination of partitioned lipids content in subcellular fractions of the brain and liver of rats at various ages are conducted. It has been found that structural maturation and myelination in rat was very advanced by 15-20 days of age (almost complete) as compared to the newborn. There is a progressive increase in the total lipid content per dry weight of the tissue which approximates 25% at 5 days and about 34% at 30 days of age in respect to the brain of a newborn. Other studies are in progress. Also, preliminary observations indicate that relative proportions of saturated to polyunsaturated fatty acids change substantially during the course of maturation.

2. Aqueous extractions of acid mucopolysaccharides of liver, brain, spleen and skin from a patient who died of Hunter-Hurler syndrome have been carried out. The mucopolysaccharides were quantitated and separated into individual components. Similar procedures were applied to the tissues of a patient who died of a non-metabolic disease. The interesting finding with this method of extraction was as follows: the predominant acid mucopolysaccharide in the liver of the patient was heparitin monosulfate (over 75%), whereas in the brain, spleen and skin, the predominant mucopolysaccharide was chondroitin sulfate B (over 75%). In normal organs both these mucopolysaccharides occur in small quantity as compared to chondroitin sulfate A and C which predominate. These observations are of importance in view of the existence of several variants of Hunter-Hurler's disease with differing ratios of heparitin sulfate to chondroitin sulfate B excreted in their urine. These ratios are quite constant for the particular variant.

Significance to Bio-Medical Research and the Program of the Institute: The composition and function of the brain during maturation and adult years, both in health and disease, are among the most important research objectives of our institute.

Proposed Course of the Project: The relevant work to this project has just begun and it is expected to continue for several years to come.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 SN/NP 1420(c)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Early Histochemical and Ultrastructural Changes in Perinatal Asphyxia.

Previous Serial Number: Same

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: Miroslaw J. Mossakowski, M.D., Ronald Myers, M. D., Don M. Long, M.D., Ph.D., Helen Rodriguez, M.D., Joseph T. Walker, Jr.

Cooperating Units: Laboratory of Perinatal Physiology.

This project has been terminated.

Serial No. NDB(I)-67 SN NP 1421 (c)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Effects of Partial Ischemia on the Nervous Tissue.

**Previous Serial Number:** Same

**Principal Investigator:** Don M. Long, M.D., Ph.D.

**Other Investigators:** Igor Klatzo, M.D., Miroslaw J. Mossakowski, M.D.

**Cooperating Units:** None.

This project has been suspended in view of the departure of the principal investigator.

Serial No. NDB(I)-57 SN/CP 401(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Psychological Evaluation of Temporal Lobe Operations

Previous Serial Number: SAME

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: M. Baldwin, M.D., A. Ommaya, F.R.C.S., P. Fedio, Ph.D., and J. Van Buren, M.D., Ph.D.

Cooperating Units: None

Man Years
Total: 1.1
Professional: 1.0
Other: .1

Project Description:

Objectives: To study neurosurgical patients with temporal lobe disorders with respect to intellectual abilities, visual and auditory perception, linguistic functions and other "personality" features.

Methods Employed: Intelligence and personality tests; aphasia, audiometric, and other specialized verbal tests; tests of visual perception. A test of "stereotypy"--the production of random movement of a stick--uses a tape punch to record a 5-minute session three times a week.

Major Findings: One of the experimental tests called the "Square-Triangle" test has been administered to over 150 patients including some of the operated cases. It involves the rapid assembling of four blocks alternately into a square and a triangular form. The preliminary analysis of the course of the perceptual learning over ten trials is encouraging in that it is the first test to show significant, but small, correlations with Mooney's Closure Faces Test, which has clearly identified a conventional right hemisphere perceptual deficit after surgery. Some other features of the Square-Triangle test are also noteworthy: 1) the relation of the scores to other measures seems relatively constant throughout the ten trials rather than manifesting the conventional attenuation during practice, 2) the relation of the scores to closure test measures is discernible only in male cases (N = 96) and not in female cases (N = 63) where the negligible correlation is reversed in sign, and

3) the relation to maximum likelihood factor scores from the Wechsler IQ test is more selective in male than female cases; in males the differential relation to a perceptual factor is clearer.

Further investigation of the relation of MMPI scores to extent of cerebral removal has been undertaken. It has been possible to construct artificial scale scores which correlate highly with the extent of temporal tissue removed; in addition, it has been possible to construct a weighted set of these questionnaire items which correlates highly with extent before operation and then reverses after operation (e.g., from +0.7 to -.6). These methods of item analysis are being applied to other groups tested a year or more after surgery and the relation of these new sets to conventional MMPI scales will be determined.

Significance to Bio-medical Research and the Program of the Institute: With careful selection of patients, relations between psychological test scores and extent of cerebral lesion can be established selectively for different loci and for each sex; these relationships have implications for physiological concepts of "higher mental" processes.

Proposed Course of Project: Further development of computer programs to analyze psychometric test results, publication of findings.

Honors and Awards: None

Publications:

Lansdell, H., and Polcari, A. R.: The meaning of the Taulbee-Sisson configurational score on the MMPI with neurosurgical patients. J. Clin. Psychol. 24: 216-219, April, 1968.

Lansdell, H.: Effect of extent of temporal lobe ablations on two lateralized deficits. Physiol. and Behav. 3: 1968 (in press).

Lansdell, H.: Evidence for a symmetrical hemispheric contribution to an intellectual function. Proceedings of the 76th Annual Convention of the American Psychological Association, 1968 (in press).

Lansdell, H.: Effect of extent of temporal lobe surgery and neuropathology on the MMPI. J. Clin. Psychol. 1968 (in press).

Serial No. NDB(I)-63 SN/CP 1032(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Psychological Effects of Subcortical Lesions Used for Relief from Abnormal Movements.

Previous Serial Number: SAME

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: J. Van Buren, M.D., Ph.D., A. Ommaya, F.R.C.S., P. Fedio, Ph.D. and L. Weinberg, M.A.

Cooperating Units: None

Man Years
Total: .9
Professional: .5
Other: .4

Project Description:

Objective: In comparison with the effects of other forms of neurosurgery, to investigate the impairments resulting from subcortical lesions and electrical stimulation with the aim of delineating subcortical from cortical factors in behavior.

Methods Employed: A battery of tests [the same as those used in Project Serial No. 401(c)] is given to patients before, two weeks after, and again a year or more after operation.

The object-naming task of Project 1033(c) has been employed to test patients during stimulation through the intracranial electrodes in the thalamic and other subcortical areas. In addition to the standard naming procedure, the test incorporated a motoric and delayed-recall feature. In this form, the task permitted an analysis of arrest errors and those related to dysnomia, a memory impairment and loss of consciousness.

Major Findings: No further results have been produced this year with regard to data from psychometric assessments; sufficient data seem to be at hand, however, to warrant analysis in the coming year.

The effect of electrical stimulation on short term verbal memory was studied in 17 patients with thalamic target electrodes and 5 patients with temporal lobe electrodes. Stimulation sites were plotted in the lateral thalamus, and parietal and temporal white matter in the left and right brain. Performance trials were selected to exclude naming errors, changes in speech or consciousness, subjective feelings and ictal events associated with stimulation.

Stimulation in the left pulvinar produced significant disruption in short term verbal memory. A moderate degree of impairment was associated with stimulation in the left and right parietal white matter. Stimulation in the right pulvinar and in the left lateral thalamus (outside the pulvinar) did not impair memory performance. No memory deficit was recorded during stimulation in the left and right temporal white matter.

The anatomic sites associated with recall disturbance are the same subcortical areas in which anomic behavior was produced in a previous study. An asymmetry in the organization of verbal processes appears to exist at the thalamic level. The data clearly demonstrate left pulvinar participation in language functions and short term verbal memory.

Proposed Course of Project: Analysis of the psychometric data.

Honors and Awards: None

Publications:

Ojemann, G. and Fedio, P.: Short term memory and stimulation in the thalamus and the parietal and temporal white substance in man. J. Neurosurg. 1968 (in press).

Serial No. NDB(I)-63 SN/CP 1033(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Phonemic Aspects of Dysnomia

Previous Serial Number: SAME

Principal Investigators: Herbert Lansdell, Ph.D. and Paul Fedio, Ph.D.

Other Investigators: L. Weinberg, M.A.

Cooperating Units: None

Man Years:
Total: .1
Professional: .1
Other: .0

Project Description:

Objective: To investigate the phonemic aspects of induced dysphasia and normal naming errors.

Methods Employed: Filmstrips and a projector are used. The filmstrips contain three orders of pictures; the names of the pictured objects have a wide range of phonemes in the initial and final positions. The nouns have a high frequency in the English language. The projector has an attached miniature screen; there is automatic control of exposure time from 30 to less than 0.5 sec/frame.

Major Findings: Much of the data being accumulated with these tests are considered under project nos. 1032(c) and 401(c). Data solely relevant in this project are being accumulated with its use during the carotid Amytal tests. With the limited and selected data available to date, there appears to be no discernible confirmation of the previous result regarding the influence of the initial and final phonemes on naming errors; the nature of the word substitutions unfortunately cannot be investigated with the present small number of observations.

Significance to Bio-medical Research and the Program of the Institute: Future different types of data may continue to extend our knowledge of the phonemic aspect of speech disruption and to delineate this clue about the physiological mechanisms of speech.

Proposed Course of Project: The apparatus will be used in these projects and the phonemic aspect of the results will be analyzed when sufficient data are obtained.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-66 SN/CP 1245(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: EEG Learning Correlates using Scalp and Intracranial Depth Electrodes.

Previous Serial Number: SAME

Principal Investigators: Paul Fedio, Ph.D. and William H. Sheriff, M.A.

Other Investigators: J. Van Buren, M.D., Ph.D., A. Ommaya, F.R.C.S., M. Baldwin, M.D., M. Buchsbaum, M.D., J. Bryan, M.A., and M. Haddox, R.N.

Cooperating Units: Laboratory of Psychology, NIMH

Man Years
Total: .2
Professional: .1
Other: .1

Project Description:

Objective: To study the neural correlates of perception, information storage and retrieval during learning.

Methods Employed: An auditory list of paired-associates is used as the learning task. During the testing procedure, simultaneous EEG is recorded from an electrode situated in the medial temporal or thalamic region in patients with epilepsy or motion disorders. Control periods contain resting levels and the stimulus-response characteristics of the learning session. Autocorrelograms and spectral analysis of the EEG data for the learning stages and control periods are computed.

A spatial maze is also used and requires that the subject traverses with a stylus a predetermined path from a start position to a goal. During performance, correct and incorrect choices are signaled by two distinct tones. Prior to testing, the same tones are used to determine the subjects' baseline responsivity. Evoked responses are calculated for each signal during baseline and testing conditions.

Major Findings: Preliminary analysis suggests that power spectral treatment appears to be too gross an index of electrographic activity which

may accompany learning.

Maze performance by patients with electrodes situated in the thalamus (200(c)) or cingulum (1424(c)) yielded differential evoked responses which correlate with correct or incorrect performance. Evoked responses recorded from the right pulvinar showed consistent and reliable waveforms, most notably for error responses. No evoked pattern was recorded from other sites in the right thalamus or caudate leads. In one patient with bilateral cingulum electrodes, an evoked response was elicited from both cinguli, again, in association with error performance. In each instance, the evoked response accompanying correct performance was less distinct than that for incorrect responses.

Significance to Bio-medical Research and the Program of the Institute: Data available from patients following temporal lobectomy show a significant learning deficit. The technique employed in this project affords a more precise method for examining the role of neural structures during the course of learning.

Proposed Course of Project: To employ various learning tasks and develop programs for EEG data analysis.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 SN/CP 1424(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes with Amygdaloid and Cingulate Lesions.

Previous Serial Number: SAME

Principal Investigators: Paul Fedio, Ph.D. and Ayub Ommaya, F.R.C.S.

Other Investigators: Theodore Zahn, Ph.D., William Sheriff, M.A., M. Haddox, R.N., and Lucy Weinberg, M.A.

Cooperating Units: Laboratory of Psychology, NIMH

Man Years
Total: .2
Professional: .1
Other: .1

Project Description:

Objective: Various physiological and electrophysiological changes in cognitive and emotional functions will be studied in relation to electrical stimulation (or recording) through indwelling cerebral electrodes and also in relation to limbic lesions.

Methods Employed: Pre- and postoperative changes in the 'appreciation of pain' will be evaluated by psychophysiological correlates and evoked responses. Behavioral measures of set and attention (reaction time) and visually guided behavior (spatial maze test) will be recorded. EEG learning parameters using intracranial depth electrodes will be obtained. The study will also evaluate the effects of intracranial stimulation on various verbal and nonverbal tests of perception, short term memory and storage.

Major Findings: The effects of stimulating bilateral cingulum electrodes were studied during the presentation of a serial task of naming and short term memory. Whereas stimulation in the left thalamus, specifically the pulvinar, produced anomia (200(c)), cingulum stimulation failed to modify naming behavior. In contrast, a greater memory disruption was associated with left cingulum stimulation than with left pulvinar stimulation. No appreciable changes in short term verbal memory were observed from right cingulum (and

right pulvinar) stimulation.

Preoperative and postoperative physiologic evaluation of 5 patients with intractable pain showed a marked decrease in 3 cases in electrodermal activity following cingulumotomy. In the remaining cases, 1 patient showed a moderate GSR decrease, 1 patient showed no change. These measures also appear to be related to independent observations regarding changes in pain perception.

Significance to Bio-medical Research and the Program of the Institute: This project provides an opportunity to define precise behavioral and autonomic concomitants of limbic functions in man. The observations also contribute to a better understanding of the mechanisms related to modifications in pain apperception following cingulumotomy in patients with intractable pain.

Proposed Course of Project: To continue the study and to extend the protocol to include patients subject to temporal lobectomy.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1967 through June 30, 1968
Branch of Ophthalmology, Intramural Research
National Institute of Neurological Diseases and Blindness

Ludwig von Sallmann, M.D., Chief

Major changes have taken place in the ranks of the laboratory investigators of the Branch and in the organization of the sections during the last few months. The outstanding event was the appointment of Dr. M.G.F. Fuortes as Chief of the newly created Laboratory of Neurophysiology, NINDB. Dr. Fuortes had been Head of the Section on Ophthalmology Physiology for more than ten years and his departure from the Branch is deeply felt. The eminent stature of Dr. Fuortes as a scientist and leader has been acknowledged by all members of the staff throughout the years. The fundamental importance of his work on the initial electric events in the visual receptor cell has been realized far beyond the confines of the Branch, NINDB, and NIH. As the admiration of Dr. Fuortes' investigations is shared by all of us, so is the gratitude for his advice and guidance which he provided so readily. His successor as Head of the Section is Dr. Peter Gouras, whose many valuable contributions in defining the function of photoreceptors and their interactions are widely known and appreciated. Dr. Arnaldo Lasansky became the Head of the new Section of Cell Biology. As shown in cooperative work with Dr. Fuortes, his competence in electron microscopical and electrophysiological techniques make him especially suited for the tasks of this Section. It has been recently announced that another new section, Experimental Embryology, will be added to the Branch. The Head is Dr. Alfred Coulombre. This phase of the reorganization is viewed with great anticipation since the well recognized research accomplishments of Dr. Coulombre and his co-workers deal with basic problems in experimental embryology of the eye and, therefore, are expected to provide broad areas of common interest with other Sections of the Branch. The former Section of Cell Biology has been renamed the Section on Ophthalmic Metabolism and is headed by Dr. Paul O'Brien.

During the period from May 1, 1967 to May 1, 1968, 156 patients were admitted to the nursing unit for a total of 7480 inpatient days. The number of out-patients participating in our projects is listed as 736. This group of patients required 2256 visits to the Clinic. Consultation requests from other departments exceed those received in other branches as 1612 such requests were answered by the ophthalmology service.

In the Sections of Ophthalmology Physiology and Cell Biology it was possible to record across the photoreceptor membrane in the leech and in this way to provide for the first time evidence that this structure generates the receptor potential. Electronmicrographs demonstrated that the photoreceptor membrane surrounding a large vacuole is an infolding of the cell surface. Thus, in the vacuole, an extracellular potential could be recorded with microelectrode and its polarity compared with the intracellularly recorded receptor potential. The position of the microelectrode in different compartments of the same cell was demonstrated by the iontophoretic introduction of a dye marker.

In limulus the photoreceptor membrane is exposed to the extracellular medium since tracer experiments with horse radish peroxidase showed minute interstices between the microvilli of the rhabdom serving effectively as diffusion pathways.

A study on the effect of oxygen deprivation on the responses of visual cells to light stimuli demonstrated that anoxia abolishes visual responses. A complicating factor was the decrease of the membrane potential under these conditions. Thus, the question of whether oxygen is needed for the chemical reaction elicited by exposure to light or for maintaining the normal electrical properties of the cell membrane could not be answered.

In the rhesus monkey eye, each of the three cone mechanisms were identified in single ganglion cells. Two types of on-center cells have been recognized in the central retina. One type receives signals from the red and green sensitive mechanisms, both of which excite in the center and inhibit in the periphery of the cell's receptive field. Signals of the blue sensitive cones cannot be detected in these cells. The cells discharge transiently to maintained stimuli of any wavelength and are called phasic. The second type of cells receive excitatory signals from only one cone mechanism, either red, green or blue-sensitive, in the center of the receptive field and inhibition from another cone mechanism in the periphery of its receptive field. These cells discharge continuously to maintained stimuli of appropriate wavelength and are called tonic. Tonic cells outnumber phasic cells although both types are found adjacent to each other throughout the retina. Phasic cells are relatively more numerous toward the periphery and tonic cells are relatively greater in number toward the fovea. Tonic cells seem to belong to the midget cell system of Polyak.

The Section on Ophthalmic Chemistry has been engaged in the study of the chemistry of rhodopsin. Extraction with a non-ionic detergent followed by chromatography on ECTEOLA-cellulose and calcium phosphate has produced a highly purified protein showing a single band on polyacrylamide gel electrophoresis, behaving as a single homogeneous component in the analytical ultracentrifuge, and showing the highest spectral purity ever reported. Although bleaching had no effect on ultracentrifugal behavior, it did produce a considerable change in the ultraviolet absorption spectrum and a very significant loss of helical structure as indicated by optical rotatory dispersion and circular dichroism studies. A minimum molecular weight of 30,000 per mole of retinal was estimated from the amino acid composition. Molecular weight studies on succinylated preparations suggest that rhodopsin exists as a polymer of, as yet, undetermined size. The conformational changes produced by bleaching of this molecule may provide a clue to understanding the mechanism of visual excitation.

In studies of model systems pertaining to transparency and opacity of gels, three proteins were used. Bovine β-lactoglobulin-A was found to exhibit an indefinite self-association. Alpha-1-crystallin from bovine lenses showed two major components with molecular weights of 850,000 (80%) and 3,500,000 (20%) after chromatography on DEAE cellulose. Ultracentrifugal analysis indicated a reversal of the proportions of these two molecular species suggesting a monomer-tetramer relationship. Succinylation of both fibrin and fibrinogen

resulted in modification in both structures such that new sites susceptible to the proteolytic action of thrombin were exposed. The ability to study and modify the interactions between gel-forming protein units will lead to an understanding of the physical and chemical aspects of the formation of transparent gel, such as those of the lens.

In related studies, the physical and chemical parameters of soluble vitreous humor proteins have been investigated. Using a procedure developed for the isolation of serum glycoproteins, a single protein was isolated by column chromatography. Ultracentrifugal analysis revealed a major component of 16,000 (80%) and a minor component of 90,000 (20%) molecular weight. The association of these species or subunits into a single component is indicative of the type of interaction involved in the structure of the vitreous body.

Oxidative metabolism of the pigment epithelium was studied with particular attention to the role of the endoplasmic reticulum in retinal-retinol interconversion and melanin formation. The microsomal haemoprotein P-450 was found to be present and was identical with a haemoprotein previously isolated from heart microsomes. Cytochrome $b_5$ was not detected. Since the haemoprotein could be reduced by pyridine nucleotides and was readily auto-oxidized, it could function as the terminal oxidase of the pigment epithelium microsomes, regulating the conversion of retinol to retinal. In addition, this haemoprotein was rapidly reduced by pyridine nucleotides in the presence of DOPA, a possible intermediate in melanin formation. It is possible that the pigment epithelial endoplasmic reticulum, which is in part transformed into melanin granules, contains the enzyme system to produce melanin, with haemoprotein P-450 as an integral part of the oxidative pathway. In addition, P-450 in other tissues is involved in drug metabolism and may be of significance in the effect of drugs on the pigment epithelium.

The Section on Ophthalmic Metabolism has studied the control of processes involved in the synthesis of glycoprotein in the bovine retina. A key enzyme in the metabolism of amino sugars, precursors of the carbohydrate residues of glycoproteins, has been shown to be under strict feedback control by an amino sugar nucleotides, one of the end products of the metabolic pathway. The control mechanism is unique in that slight increase in the intracellular level of the amino sugar nucleotide alter the enzyme in such a way that it becomes sensitive to inhibition by glutamine, one of the substrates. The maximum inhibition occurs at physiological levels of glutamine. A slight rise in the end product concentrations is thus amplified by coupling its effect with a normally high level of glutamine. The result is a very efficient control mechanism which prevents overproduction of these glycoprotein precursors. The sequential addition of sugars to the protein care of glycoproteins takes place in the microsomal fraction of the retina. Kinetic studies of the sugar transfer enzymes indicate that very low levels of sugar nucleotide precursors are sufficient for maximum rates of glycoprotein synthesis. Thus, the entire sequence of amino sugar metabolism and incorporation into glycoprotein is a highly efficient organized system.

The incorporation of radioactive amino sugars and amino acids by whole retinas has also been studied *in vitro*. Initially, the microsomes become labeled.

Subsequently, the mitochondria and photoreceptor outer segments become progressively more highly labeled. Considerably more protein synthesis takes place in dark-adapted eyes. Thus, directly or indirectly, light exerts some control of the overall process of protein synthesis, including glycoproteins, in the photoreceptors.

In the Section of Ophthalmology Pharmacology the cat's intact living eye or the arterially perfused enucleated eye were used as experimental models. Iris-ciliary body, and iris preparations were also isolated for investigation of drug effects on intraocular vessels. An array of autonomic drugs, adrenergic or cholinergic in nature, and blockers of such compounds were examined regarding their function in the intraocular pressure, the iris artery pressure, and the perfusate flow rate through this artery. Moreover, experiments were designed which allowed estimation of the influence of autonomic drugs on the rate of aqueous humor formation. In this part of the work it was observed that under the conditions of the experiment, eserine + acetylcholine, isoproterenol, and unexpectedly, l-epinephrine increased the rate of aqueous humor inflow. Reports in the literature state that l-epinephrine decreased the inflow rate. In view of this contradiction between results of various laboratories it is planned to repeat the experiments by using a different method of measuring inflow rate.

In another study in this Section it was attempted to analyze the mechanism of action of pharmacologic agents which constrict the iris artery of the cat. The action of isoproterenol (a beta adrenergic stimulator) was inhibited by an alpha adrenergic blocker (tolazolin) as well as by the parasympathomimetic block of atropine. Two beta blocking agents (pronethalol or propranolol) inhibit the action of several pharmacologic agents, among them, eserine, epinephrine, isoproterenol, and acetazolamide. It is not known at present to which extent the responses of the iris artery to these drugs in the cat is associated with changes in the intraocular pressure in this species or in the human eye.

Buphthalmic rabbits have been made available for investigations of this pathologic condition which resembles hydrophthalmos in children. The present study is based on the concept that the outer coats of buphthalmic rabbit eyes cannot withstand the stretching force of the intraocular pressure. Increase of the anterior chamber volume was interpreted as caused by the augmented elasticity of the cornea and possibly the sclera when compared with reaction to stress of these tissues in normal eyes. It is questionable, therefore, that the pathophysiologic changes in infantile glaucoma in humans are similar to those reported in buphthalmic rabbits.

In the general area of histopathologic studies in cooperation with the Laboratory of Perinatal Physiology an examination was carried out of the ocular abnormalities present in neonatal rhesus monkeys that had been subjected to either bilateral ligation of the carotid arteries and jugular veins or transient compression of the umbilical cord at the middle of the gestation period. The most frequently observed lesion was an extensive cystic degeneration of the retina leading to retinoschisis. Defects of the anterior portion of the eye included leukoma adhaerens and anterior polar cataract complicated by the attachment of a dense persistent pupillary membrane. There was a marked

resemblance between the lesions of the monkey eye developing subsequent to induced fetal hypoxia and well-known congenital abnormalities of the human eye.

Pathogenetic aspects of the vasculo-occlusive retinopathy occurring in conjunction with experimental allergic encephalomyelitis in monkeys has been restudied in a short series of experiments. In the allergic response of the central nervous system to inoculation with guinea pig spinal cord antigen a heavy mantle of granulomatous inflammation is commonly found about the axial vessels of the optic nerve. In two eyes of this series occlusion of the central artery of the optic nerve was demonstrated as well as congestion with hemostasis in the central vein. This observation suggests that disturbance of the circulation in these vessels precipitated the hemorrhagic retinopathy. In contrast to the vasculo-occlusive disease of the retina following the administration of spinal cord antigen, monkeys sensitized to homologous retina developed retinal perivasculitis and uveitis without accompanying neurological deficits. While the relative involvement of choroid and retina varied considerably in some animals the lesions in the uvea were minimal or absent and in these cases the retinal periphlebitis was considered to be a direct autoimmune lesion. Bovine retina, homologous choroid plexus, and kidney of guinea pig did not evoke an allergic response in the monkey eye.

Successful malignant transformation of ocular tissue with oncogenic DNA viruses in vitro has been accomplished for the firsttime in a joint effort with investigators of the Pathologic Anatomy and Viral Biology Branches of NCI. Transformation was achieved in cultures of cells derived from hamster iris, choroid, and retina and the transformed cells produced tumors when injected into irradiated hamsters. All of the ocular tissue transformed with SV40 virus and produced neoplasms composed of epithelioid and spindle-shaped cells with large nuclei. Choroid and iris, transformed with the hybrid virus LLE46 produced tumors similar to the SV40 type while retina infected with LLE46 gave rise to tumors which were bimorphic, containing both SV40 type cells and small hyperchromatic cells resembling an adenovirus-induced tumor. Polyoma virus and adenovirus 12 transformed only retina and the latter agent produced tumors which had some resemblance to retinoblastomas although no rosettes or neural elements could be identified.

A study of the possible injury to the eye resulting from scleral transmission of laser light has been concluded with the evaluation of the extent and type of lesions produced in living rabbits by ruby and neodymium laser beams directed through the sclera and ciliary body and passing just behind the lens. Dosage related chorioretinal destruction beneath the site of beam impact was accompanied by vitreous coagulation, hemorrhage and secondary retinal detachment. Various secondary changes involved the anterior segment depending on the site and extent of the initial lesion. They included corneal vascularization, formation of a retrocorneal membrane, hemorrhage and focal muscle necrosis. In a number of eyes posterior subcapsular opacities of unique configuration were produced. These were of a symmetrical elliptical shape with the widest portion of the opacity at the posterior pole. The location and form of the cataract suggests local energy absorption with consequent necrosis of the affected fibers.

Capillary permeability in the iris and ciliary body of the mouse eye has been investigated by electronmicroscopic examination of the fine structure of the vessels and the transport of an electron-dense tracer through the vessel walls and across the ciliary epithelium. Iris capillaries demonstrate short tight junctions between endothelial cells which prevent the transport of intravascular horseradish peroxidase into the iris stroma. The endothelium of ciliary body capillaries, in contrast, show closed fenestrae, and the tracer escapes rapidly from the vessels and subsequently through both layers of the ciliary epithelium to the anterior chamber. The mode of transport through the ciliary epithelium cells is as yet undefined.

Continuing investigations of the cause of the stimulation of mitotic activity in the rat lens epithelium during the early phase of sugar cataract development have demonstrated that the close temporal relation of lens swelling and mitotic stimulation characteristic of galactose cataract does not exist in the case of alloxan-diabetic cataract. In the latter case mitotic stimulation occurs without a measurable increase in lens volume. In organ-culture experiments designed to define the role of galactose-induced lens swelling as a stimulus to cell proliferation failed because of the severe toxic effects of galactose on the lens epithelium under these conditions. Mitotic activity is suppressed even in the presence of low concentrations of galactose. Gradual increase of the tonicity of the medium during the incubation period could prevent the damaging effects of galactose demonstrating that, in vitro, they are due to an osmotic imbalance of the epithelial cells.

Studies of the normal rat lens maintained in a basic medium revealed a gradual reduction of mitotic activity over a period of two days with a more drastic effect on the differentiating cells of the meridional rows. This portion of the population showed early cell death and subsequent disorganization. The addition of small amounts of serum or insulin to the medium prevents the occurrence of cell destruction but the population of the rows is reduced after 48 hours suggesting impairment of the flow of cells from the proliferating to the differentiating compartments.

The toxic effects of iodoacetate and chloroquine on the cat retina were studied by eliciting electrophysiological responses and comparing these with ophthalmoscopic findings and histopathologic changes. The two agents were either injected into the vitreous or were administered through the posterior ciliary artery. When injected intravitreally both drugs depressed the two main components of the electroretinogram. The toxic effect of iodoacetate was also recognized when injected into the posterior ciliary artery. Chloroquine administered in this way did not influence the function of the retina on electroretinographic testing. Histopathologic examination of these eyes has not been completed.

New information has been gained in clinical studies, predominantly in three disease groups, retinal degeneration, uveitis, and glaucoma. Examination of patients with recently developed methods was coordinated with or based on results of laboratory studies.

Electrophysiological and psychophysical examination of patients with various forms of retinal degeneration were conducted on the offspring of families afflicted by retinitis pigmentosa before the characteristic features of the disease had developed. With the Ganzfeld technique and balanced light stimulation, deficits of the rod and cone components in the electroretinogram could be differentiated and correlated with different genetic types of the disease entity. Such family studies were conducted in patients with dominant retinitis pigmentosa, sex-linked retinitis pigmentosa, a recessive cone-rod degeneration, dominant retinitis pigmentosa with incomplete penetrance and dominant cone degeneration. The complex of elaborate electrophysiological and psychophysical tests used in these examinations do not lend themselves to a brief review but it can be said that the combination of the tests contributed to the understanding of underlying defects in retinal degenerations. The inclusion of early and atypical case of retinitis pigmentosa with genetic information furthered the analysis and clarification of various types and allowed a limited prognostic evaluation.

In the uveitis project the results of antimetabolite therapy in various forms of the disease were reported and the occurrence of serious side effects was emphasized. Methotrexate or cytoxan were the immunosuppressive agents employed in about 30 patients. The course of the disease in patients with several types of uveitis was influenced beneficially even after prolonged steroid treatment has been proven ineffective. However, as a rule relapses of the inflammatory process were not prevented and some patients with severe uveitis, including sympathetic ophthalmia, Behcet's disease, and uveal effusion syndrome did not benefit from the administration of these antitumor drugs. A final evaluation of their usefulness, particularly in combination with steroids must be postponed until the results of prolonged therapy with these drugs becomes available.

Of great interest were the laboratory studies on the patients treated with the immunosuppressive drugs. Quantification of immunoglobulin levels prior to and after termination of the therapeutic course, the effect of treatment on the primary immune response, studies of the drug induced changes in the induction of delayed hypersensitivity and on the cytologic response established with the skin window technique elucidated several facets of the mechanism of drug action. It was concluded that most likely anti-inflammatory properties of these agents and not immunosuppression was responsible for the encouraging results when obtained.

A crystallographic analysis carried out by Dr. P.D. Frazier on eye tissues of patients with cystinosis provided new information on the nature of the crystals observed in clinical and routine histologic examinations. Spindle-shaped or fusiform bodies were embedded in the cornea and sclera, and a second type of crystal of rectangular shape was found in the conjunctiva and uvea of such patients. X-ray diffraction studies allowed to identify the rectangular forms as l-cystine. The crystals in the cornea could not be characterized by their diffraction patterns. Probably they are not l-cystine crystallites. Micro-amino acid analysis confirmed the nature of the rectangular deposits as being l-cystine but failed to identify the composition of the spindle-shaped bodies.

Several studies which deal with the glaucoma project are under way but they are not in a state to allow evaluation. A valuable contribution was made in this report year in the systematic studies of the pressure in the episcleral vein with the aid of a newly designed instrument, a modified force displacement transducer with a lucite cone applanation tip. The pressure measurement in these veins with this instrument is greatly facilitated as it can be mounted on a slit biomicroscope and allows the determination of the end point, that is collapse of the vein, by the reading on the drum the pressure necessary to interrupt the circulation in these vessels. In two members of a family with hereditary glaucoma, the episcleral veins were grossly dilated and the pressure was found to be significantly increased. In these patients the effect of the increased pressure in the episcleral veins, into which the aqueous veins feed, on the intraocular pressure was well demonstrated.

The research activity of the Branch is reflected by the publication or acceptance of 40 manuscripts during the report period. Three projects were completed, three were terminated due to organizational changes, twenty-five were continued and seven new projects were added.

Serial No. NDB(I)-56 O/OPS 300(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Design and Construction of Ophthalmic Instruments; Research in Psychophysical Methods of Evaluating Vision

Previous Serial Number: SAME

Principal Investigator: Ralph D. Gunkel, O.D.

Other Investigators: Peter Gouras, M.D.
Eliot L. Berson, M.D.

Cooperating Units: Medical Arts and Photography Branch, DRS

Man Years

| | |
|---|---|
| Total: | 0.6 |
| Professional: | 0.6 |
| Other: | 0.0 |

Project Description:

Objectives: Efforts are made to secure maximum effectiveness of instruments, techniques and ideas from whatever source for use in clinical and experimental work relating to ophthalmology. Modification of conventional instruments and design and construction of new ones is often warranted.

Methods Employed: Frequent additions and modifications are needed in the apparatus used in psychophysical and electrophysiological studies of the eye. Clinical Associates often require new or different instruments in their research projects, and occasional repairs must be made due to use or misuse of even the conventional instruments.

Major Findings: Measurements of localized rod and/or cone sensitivity were made on nearly all of the 297 patients who had dark adaptation studies during the past year. These included the studies of toxic retinopathies, tapeto-retinal degeneration, and non-progressive congenital defects. This work is a substantial part of three papers which have been accepted for publication in the July Archives of Ophthalmology. Chief author, Dr. Eliot Berson will discuss these data at greater length in his report.

A double interference spectral wedge was incorporated in a slide fitting the Goldmann-Weekers adaptometer, thereby greatly increasing the potential for spectral sensitivity measurements.

The Ganzfeld technique for electroretinography has been further refined and several improvements were made in the apparatus of Dr. Gouras for electrophysiological studies. A new stimulator was constructed incorporating a 17-ampere tungsten filament lamp, condensing system, shutter and filter cells.

A pressure-transmitting cone was constructed which permits clear viewing of scleral vessels while Dr. Podos measures the pressure within them for a glaucoma study.

Some contributions have been made to the newly-acquired pupillograph.

The Polaroid adapter for fundus photography has been redesigned to eliminate the need for the 2X attachment and give greater enlargement. The present light was found to be adequate for the most pigmented retina and the new 73-millimeter colored photographs are now routine for the medical records.

Intermittent work on the model eye has continued, with the addition of a "crystalline lens" and consequent redesign of both anterior and posterior segments to conform more closely to a plaster mold of an actual human eye. It is felt that the practical limits to perfection will soon be attained and a few model eyes must be produced for use in demonstration, instruction, and instrument application.

Significance to Bio-medical Research and the Program of the Institute: This project has contributed substantially to the efficacy, the continuity and the published results of several projects within the Ophthalmology Branch.

Proposed Course of Project: It is proposed that the project be continued in its present flexible form.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-60 O/OPS 700(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Activity of Visual Receptor Organs

Previous Serial Number: SAME

Principal Investigator: M.G.F. Fuortes, M.D.

Other Investigators: Antonio Borsellino, Ph.D.
Anthony Gorman, M.D.
John McReynolds, M.D.
Gerald Wasserman, Ph.D.

Cooperating Units: Instituto de Fisica, University of Genoa, Genoa, Italy
Laboratory of Neuropharmacology, NIMH
Marine Biological Laboratory, Woods Hole, Massachusetts

Man Years

| | |
|---|---|
| Total: | 3.0 |
| Professional: | 1.5 |
| Other: | 1.5 |

Project Description:

Objectives: The aim of the present research is to identify the processes responsible for activation of visual receptor cells.

Methods Employed: Research has been performed using the same electrophysiological methods as in previous years.

Major Findings: Work on responses of visual cells of Limulus to single photons has been completed. The results of this analysis have been reported in two articles which have now been accepted for publication.

In an effort to study the role of metabolism in visual responses, experiments have been performed in order to determine how oxygen deprivation affects the response of visual cells to illumination. It has been found that the visual response disappears in the absence of oxygen. In these conditions, however, the membrane potential decreases. Due to this complication, it is not clear whether oxygen is required for the chemical reactions initiated by light or for maintaining the normal electrical properties of the cell's membrane.

A study has been started on the relations between changes of membrane potential and impulse firing in eccentric cells of Limulus. The results obtained so far indicate that if depolarization is decreased rapidly, firing frequency diminishes drastically whereas the decrease of frequency is less pronounced if the same change of polarization is brought about slowly. These results suggest that the message which the visual cell transmits to the central nervous system emphasizes changes of illumination.

Responses of visual cells of Limulus to lights of different wavelengths have been investigated. Two types of cells have been found: the majority (called "alpha cells") have an action spectrum which corresponds well with the absorption spectrum of rhodopsin; a smaller group of cells (called "beta cells") has an action spectrum extending to the near ultraviolet. It could not be determined whether beta cells contain two different pigments. This work on action spectra will be continued by Dr. Wasserman at the University of Wisconsin.

Significance to Bio-medical Research and the Program of the Institute: Study of the basic mechanisms leading to responses of visual cells is important for understanding of the processes responsible for the specific actions of receptor cells. The conclusions reached in previous years have been confirmed in the recent work and the general theories proposed proved to be useful for interpreting the mechanisms of visual responses.

Proposed Course of Project: The projects mentioned above will be continued in the Laboratory of Neurophysiology, NINDB.

Honors and Awards: None

Publications:

Borsellino, A. and Fuortes, M.G.F.: Responses to single photons in visual cells of Limulus. J. Physiol. (in press), 1968.

Borsellino, A. and Fuortes, M.G.F.: Interpretation of responses of visual cells of Limulus. Proceedings of the Institute of Electrical and Electronics Engineers. (in press), 1968.

Serial No. NDB(I)-63 O/OPS 1012(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electrophysiologic and Psychophysical Studies of Retinal Degenerations

Previous Serial Number: SAME

Principal Investigators: Peter Gouras, M.D.
Eliot Berson, M.D.

Other Investigators: Ralph D. Gunkel, O.D.

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.8 |
| Professional: | 1.3 |
| Other: | 0.5 |

Project Description:

Objectives: To study retinal degenerations in man with electrophysiological and psychophysical methods.

Methods Employed: Dark adaptation and color vision testing, retinal profiles, perimetry, electroretinography and electro-oculography are used to study patients and their families. In addition, retinal degenerations have been studied with fluorescein fundus photography.

Patient Material: The subjects are either outpatients or are admitted to the in-service unit; they are referred from other institutes of NIH and from private ophthalmologists. Normal subjects were obtained from the Normal Control Program of NIH.

Major Findings: Families of patients with retinitis pigmentosa have been examined to study the early psychophysical and electrophysiological changes in affected off-spring before pigmentary migration and marked destruction of the retina have occurred. Using the ganzfeld technique of electroretinography, developed at NIH, definition of the cone and rod components in the electrical recordings of a variety of degenerations has been accomplished. These findings have been correlated with the different genetic types. Studies have been conducted on dominant retinitis pigmentosa with incomplete penetrance, and dominant cone degeneration.

The ganzfeld system has allowed a better understanding of the timing of cone and rod responses in these recordings. Reduction in amplitude as well as delay in implicit times of rod and cone components have been detected in generalized retinal diseases while only amplitudes are changed in patients with focal chorioretinal scars.

Retinal degenerations with electrophysiologic defects in the rod or cone system remain untreatable. An attempt to alter these responses by light exclusion for a short period in selected children was unsuccessful. Two degenerations, sex-linked retinitis pigmentosa and sex-linked choroideremia were studied on an inpatient basis but no associated systemic metabolic defect could be found in these conditions.

Fluorescein fundus photography suggests that the electrophysiologic and psychophysical abnormalities precede any alteration in fluorescein flow through retinal or choroidal circulation.

Significance to Bio-medical Research and the Program of the Institute: The underlying defects in retinal degenerations will be better understood through study of early or atypical cases and through genetic classification of various types. The finding of latency as well as amplitude changes in rod and cone components offers a new parameter in the consideration of these defects. Clarification of the primary defects in the rod and cone receptors is essential for defining the mechanisms of these degenerations.

Proposed Course of Project: Patients with various retinal degenerations will continue to be examined using the more sensitive electroretinography apparatus with special attention to early diagnosis in family studies and to atypical cases. Follow-up

examinations will be done to gain information on the course of these degenerations.

Honors and Awards: None

Publications:

Berson, E., Gouras, P., and Gunkel, R.: Rod responses in retinitis pigmentosa, dominantly inherited. Arch. Ophthal. (in press), 1968.

Berson, E., Gouras, P., and Gunkel, R.: Progressive cone degeneration, dominantly inherited. Arch. Ophthal. (in press), 1968.

Berson, E., Gouras, P., and Gunkel, R.: Progressive cone-rod degeneration. Arch. Ophthal. (in press), 1968.

Finkelstein, D., Gouras, P., and Hoff, M.: Human electroretinogram near the absolute threshold of vision. Invest. Ophthal. 7: 214-218, Apr. 1968.

Serial No. NDB(I)-63 O/OPS 1016(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electrophysiological Studies of Mammalian Retina

Previous Serial Number: SAME

Principal Investigator: Peter Gouras, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.4 |
| Other: | 0.3 |

Project Description:

Objectives: To determine the cells responsible for generating the electrical activity of the retina following light stimulation and to understand their coding of visual information.

Methods Employed: Glass micropipette electrodes are introduced into the intact eye of the anesthetized rhesus monkey in order to study the responses of single neural elements. The retina and the position of the microelectrode can be observed by means of a modified fundus camera. The responses of single ganglion cells and groups of bipolar cells can be studied with small light stimuli, obtained from a dual beam optical system. The energy, wavelength, area, timing and retinal position of these stimuli can be independently controlled in both beams.

Major Findings: Red, green, and blue sensitive cone mechanisms have been isolated and studied in two types of on-center

ganglion cells in the rhesus monkey's retina. One type of cell receives signals from both red and green sensitive cone mechanisms, synergistically acting to produce excitation in the center and inhibition in the periphery of its receptive field. These cells respond transiently to diffuse stimuli of any wavelength and are called phasic. The second type of cell receives excitatory signals from only one cone mechanism, either red, green or blue sensitive, in the center and inhibition from another cone mechanism in the periphery of its receptive field. These cells respond continuously to diffuse stimuli of appropriate wavelength and are called tonic. Tonic cells outnumber phasic cells although both are found adjacent to one another throughout the retina. Phasic cells are relatively more common toward the periphery and tonic cells are more common toward the fovea.

Significance to Bio-medical Research and the Program of the Institute: An understanding of retinal function at the cellular level should prove valuable for concepts of vision and the pathophysiology of retinal disease.

Proposed Course of Project: To determine whether the tonic cells correspond to the midget ganglion cell system of Polyak by measuring their size via conduction velocity studies.

Honors and Awards: None

Publications:

Gouras, P.: The effects of light-adaptation on rod and cone receptive field organization of monkey ganglion cells. J. Physiol. 192: 747-760, Nov. 1967.

Serial No. NDB(I)-66 O/OPS 1428(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies of Color Vision

Previous Serial Number: SAME

Principal Investigator: Gerald S. Wasserman, Ph.D.

Other Investigators: M.G.F. Fuortes, M.D.

This project has been terminated.

Serial No. NDB(I)-68 O/CB 1528(c)
1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Origin of Electrical Responses in Photoreceptor Cells

Previous Serial Number: NONE

Principal Investigators: Arnaldo Lasansky, M.D.
M.G.F. Fuortes, M.D.

Other Investigators: None

Cooperating Units: Laboratory of Neurophysiology, NINDB

Man Years

| | |
|---|---|
| Total: | 2.1 |
| Professional: | 1.1 |
| Other: | 1.0 |

Project Description:

Objectives: To investigate the location of the excitable membrane and the nature of the transducing mechanisms in visual cells.

Methods Employed: Electron microscopy. Electrophysiological recording combined with techniques to identify the position of microelectrodes.

Major Findings: When this work began, it was not known whether the receptor potential originates at the photoreceptive membrane or at other areas of the visual cell surface. The second alternative would have implied that the excitation spreads away from the site where light energy is absorbed and that a relaying mechanism exists for this purpose within the visual cell. This hypothesis seemed to be supported by the apparent lack of

extracellular space in adjacency to the photoreceptive membrane in Limulus (Lasansky, 1967), and by reports from several workers stating that the photoreceptive membrane in the leech is intracellular.

The situation in Limulus has been investigated by using a protein, horseradish peroxidase, as an electron microscopic tracer. It has been found that minute interstices between the microvilli of the rhabdom provide effective diffusion pathways. Therefore, the photoreceptive membrane is exposed to the extracellular medium and could be the site of origin of receptor currents.

In the leech, the electron microscopic observations show that the photoreceptive membrane is in fact an infolding of the cell surface which encloses a large space (vacuole). The channels connecting the vacuole with the extracellular space are bridged by septate desmosomes. Within the vacuole, an extracellular receptor potential can be recorded with microelectrodes. This potential has the opposite polarity of the intracellularly recorded receptor potential. Therefore, the photoreceptive membrane of leech visual cells generates receptor currents.

Significance to Bio-medical Research and the Program of the Institute: These results provide a better understanding of the processes mediating visual excitation.

Proposed Course of Project: The electrical measurements in leech photoreceptors will be continued in order to determine the magnitude of the conductance changes evoked by light and the pathway followed by the receptor currents.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 O/CB 1529(c)
1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Fine Structure and Permeability of Iris and Ciliary Body Vessels in the Albino Mouse

Previous Serial Number: NDB(I)-67 O/CH 1430(c)

Principal Investigator: Richard S. Smith, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.90 |
| Professional: | 0.90 |
| Other: | 0.00 |

Project Description:

Objectives: To study the fine structure of the small blood vessels of the albino mouse iris and ciliary body in order to determine the normal vascular pattern and to observe the permeability of these vessels as measured by an electron-dense marker. To study the epithelium of the ciliary body and to observe transport of an electron-dense marker across the epithelium.

Methods Employed: Standard methods of tissue preparation for electron microscopy are used, including glutaraldehyde-$O_sO_4$ fixation. The normal ultrastructural appearance of the endothelium and perivascular tissue is studied. Vascular permeability is being examined by intracameral or intravascular injection of horseradish peroxidase. The possible transport of peroxidase across the ciliary epithelium is followed. Under suitable conditions, this electron-dense tracer can be visualized in thin sections of tissue prepared for the electron microscope.

Major Findings: Short tight junctions have been demonstrated between endothelial cells of the iris capillaries. These junctions prevent the transport of horseradish peroxidase into the iris stroma following intravascular injection of this tracer. The endothelium of the ciliary body capillaries shows closed fenestrae. Horseradish peroxidase escapes rapidly from these vessels. Within ten minutes after intravascular injection, peroxidase is transported across the vessel wall, through both layers of the ciliary epithelium and is seen in the anterior chamber, adhering to the surface of the iris and to the corneal endothelium. A significant amount of vesicular transport is observed in the ciliary epithelium. The question of intercellular transport is unresolved at this time.

Significance to Bio-medical Research and the Program of the Institute: Capillary permeability varies in different locations. The role of the iris vessels in aqueous humor outflow is not well defined. Administration of horseradish peroxidase by various routes will provide information on the permeabilities of iris vessels in relation to the outflow of aqueous humor. Study of the transport of horseradish peroxidase across the ciliary epithelium will be helpful in understanding the processes underlying aqueous humor secretion.

Proposed Course of Project: Additional electron-microscopic observations of the transport of peroxidase across the ciliary epithelium will be made in order to clarify the importance of intercellular passage of the tracer. This project will be completed with the departure of the principal investigator.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-66 O/CH 1289(c)
1. Ophthalmology Branch
2. Section on Cytology and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Fine Structure of Toad Retinal Capillaries

Previous Serial Number: SAME

Principal Investigator: Arnaldo Lasansky, M.D.

Other Investigators: None

Publications:

Lasansky, A.: Pathway between hyaloid blood and retinal neurons in the toad: structural observations and permeability to tracer substances. J. Cell Biol. 34: 617-626, Aug. 1967.

This project is complete.

Serial No. NDB(I)-66 O/CH 1291(c)
1. Ophthalmology Branch
2. Section on Cytology and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on the Endothelium of the Corneal Homograft

Previous Serial Number: SAME

Principal Investigator: Salvador R. Salceda, M.D.

Other Investigators: None

Publications:

Salceda, S. R.: Endothelial cell survival after keratoplasty in rabbits: the effect of storage of donor corneas. Arch. Ophthal. 78: 745-752, Dec. 1967.

This project is complete.

Serial No. NDB(I)-63 O/OCH 1017(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Physical Chemistry of Corneal Collagen

Previous Serial Number: SAME

Principal Investigator: Marc Lewis, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.1
Professional: 0.1
Other: 0.0

Project Description:

Objectives: To determine the physical and chemical parameters related to the molecular structure of corneal collagen with the view of examining them for significance in the morphology of the cornea.

Methods Employed: Collagen has been extracted from the bovine cornea and purified by conventional means. Ultracentrifugal analysis has been used to attempt to obtain the distribution of the molecular weights of collagen polymers.

Major Findings: Little progress has been made in studying the distribution of molecular weights of the collagen polymers because of the great technical difficulty of this problem. A reasonably satisfactory theoretical treatment of the problem has been available for only a few months and this still requires some modifications to make it suitable for handling the thermodynamically non-ideal behavior of these macromolecules.

Significance to Bio-medical Research and the Program of the Institute: This project represents a continued study of the relationship of the molecular structure of certain proteins of ophthalmic origin to the structure and function of the tissues of their origin. It provides a base for comparative studies of different collagens in the eye, and for studies of the role of collagen in corneal development and in corneal pathologies.

Proposed Course of Project: Continued studies on molecular weight distributions are planned, since it is felt that this information could be of significance in understanding the process of the aggregation of the

collagen monomers into polymers and ultimately forming the collagen fibril. It now appears as if the problem of thermodynamic non-ideality can be treated in a satisfactory manner and there is a reasonable probability of obtaining a useful estimate of the molecular weight distribution.

Honors and Awards: None

Publications: None

Serial No. NDB(1)-65 0/OCH 1210(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Chemistry of Rhodopsin

Previous Serial Number: SAME

Principal Investigators: Marc S. Lewis, Ph.D.
Hitoshi Shichi, Ph.D.

Other Investigators: Filadelfo Irreverre, Ph.D.
Audrey L. Stone, Ph.D.

Cooperating Units: Laboratory of Biophysical Chemistry, NIAMD
Section of Physical Chemistry, Laboratory of Neurochemistry, NIMH

Man Years:
Total: 1.6
Professional: 1.5
Other: 0.1

Project Description:

Objectives: To obtain highly purified preparations of bovine rhodopsin and to study the structural and functional aspects of the chemistry of this molecule.

Methods Employed: Rhodopsin was extracted from outer segments of dark-adapted bovine retinas with 1% Emulphogene BC 720, a nonionic alkoxypoly-(ethylene) ethanol, and purified by chromatography on ECTEOLA-cellulose and calcium phosphate. The purity of the pigment was examined by means of ultracentrifugation, polyacrylamide gel electrophoresis and spectrophotometry. Amino acid analyses were made with an automatic analyzer, circular dichroism and optical rotatory dispersion measurements were carried out with a recording spectropolarimeter, and molecular weights were studied by ultracentrifugal analysis.

Major Findings: (1) The best preparations showed the highest spectral purity ever reported with the following spectral ratios: $A_{400}/A_{498} = 0.168$ and $A_{280}/A_{498} = 1.75$. (2) The purified pigment migrated electrophoretically as a single band on polyacrylamide gel and behaved as a single homogeneous component in sedimentation equilibrium studies in aqueous detergent solution. (3) Bleaching had no significant effect upon the ultracentrifugal behavior of the rhodopsin in aqueous detergent solutions. However, optical rotatory dispersion and circular dichroism studies indicated a very significant loss

of a helical structure when the rhodopsin was bleached. A considerable change in the UV absorption spectrum was also observed with bleaching. (4) The amino acid composition of the rhodopsin was found to be in substantial agreement with previously published values; those differences which were found tended to indicate a higher degree of purity in the samples studied here. A minimum molecular weight of approximately 30,000 per mole of retinal was estimated from the amino acid composition. (5) It was not possible to determine a meaningful molecular weight for the rhodopsin in detergent solution as it exists as a rhodopsin-detergent complex under these conditions. Difficulties in determining the exact amount of the extensively bound detergent made a quantitative determination of molecular weight impractical; it did not preclude establishment of the homogeneity of the preparation. Modification of the rhodopsin molecule by succinylation of the epsilon-amino groups of the lysine residues was attempted as a means of obtaining a rhodopsin preparation which would not require detergent for solubilization. Approximately 50% of the amino groups could be succinylated with less than 10% bleaching, and resulted in an apparently water soluble preparation. Molecular weight studies on this preparation suggested that rhodopsin exists as a polymer, but the presence of some bound detergent in all preparations studied so far has created difficulty in determining the exact extent of the polymerization.

Significance to Bio-medical Research and the Program of the Institute: Very little is known about the biochemical nature of the protein moiety (opsin) of rhodopsin. The definition of the molecular characteristics of the protein moiety of the pigment is essential for the future investigation of structure and function of the photoreceptor of the retina. The present finding that rhodopsin undergoes considerable protein-conformational changes during bleaching by light is of particular significance in this connection, since the light-induced conformational change may provide a clue to understanding the molecular mechanism of the reaction directly responsible for visual excitation.

Proposed Course of Action: (1) Determination of the molecular weight of rhodopsin by physcochemical methods, e.g., ultracentrifugation. (2) Investigation of conformational requirements on the part of opsin for the formation of chromophore. (3) Determination of the amino acid sequence in the opsin molecule.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 0/OCH 1217(c)

1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Physical Biochemistry of Model Gel Systems

Previous Serial Number: SAME

Principal Investigators: Marc S. Lewis, Ph.D.
Emery T. Adams, Ph.D.
Jules A. Gladner, Ph.D.

Other Investigators: None

Cooperating Units: Laboratory of Biophysical Chemistry, NIAMD

Man Years

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|---|---|
| Total: | 0.2 |
| Professional: | 0.2 |
| Other: | 0.0 |

Project Description:

Objectives: To study the physical and chemical parameters of model systems which are pertinent for transparency and opacity of gel systems.

Methods Employed: Alpha-1-crystallin was obtained from bovine lenses, purified by isoelectric precipitation and column chromatography, and studied ultracentrifugally. Bovine fibrinogen and fibrin were modified by succinylation of the ε-amino groups of the lysine residues. The effects of thrombin treatment on these modified proteins was studied in the analytical ultracentrifuge. Bovine β-lactoglobulin-A was used for ultracentrifugal studies on self-associating systems.

Major Findings: Sedimentation velocity studies on the alpha-1-crystallin prior to chromatography showed three components with sedimentation rates of 3.9 S (5), 12.1 S (15?), and 15.5 S (80%). Chromatography on DEAE removed the light component; the two heavier components remained, but their relative proportions appeared to be reversed. Molecular weight analysis indicated the presence of 80% of a component with a molecular weight of 850,000, and 20% of a component with a molecular weight of 3,500,000. The 1:4 relationship of the molecular weights and the reversal of the relative amounts of these components following chromatography suggests the possibility of a reversible monomer-tetramer relationship. Although the ultracentrifugal experiments did not directly demonstrate the presence of a reversible reaction, this possibility must be considered as the chromatography could have modified the reactivity of the components.

Ultracentrifugal studies showed that thrombin treatment of succinyl fibrin resulted in the release of the same subunit with a molecular weight of 20,000 as was obtained with the thrombin treatment of succinyl fibrinogen. Since the succinyl fibrin had been obtained by the treatment of fibrinogen with thrombin prior to succinylation, this demonstrated that the succinylation modified the structure of the fibrin and the fibrinogen in such a way that new sites which were susceptible to the proteolytic action of thrombin were exposed.

The studies on β-lactoglobulin A were completed and analysis indicated that the data could best be described as an indefinite self-association.

Significance to Bio-medical Research and the Program of the Institute: The studies on the physical chemistry of the α-1-crystallin are of significance to understanding the relationships of proteins involved in tissue transparency. The studies on β-lactoglobulin-A have contributed to an understanding of associating systems and will continue to be of value in the studies of lens proteins. The research on fibrinogen has given a greater understanding of the physical and chemical aspects of gel formation which are related to tissue transparency as well as the mechanisms of blood clotting. Of equal importance, these studies have led to the use of succinylation as a means of solubilizing rhodopsin without detergent.

Proposed Course of Project: Studies on the lens proteins will be continued using a variety of appropriate physical and chemical techniques. The studies on fibrinogen have been completed and will be prepared for publication. The studies on β-lactoglobulin-A have been completed and published.

Honors and Awards: None

Publication: E. T. Adams, Jr. and M. S. Lewis: Sedimentation Equilibrium in Reacting Systems. VI. Some Applications to Indefinite Self-Associations. Studies with β-lactoglobulin-A. Biochem., 7: 1044-1053, March, 1968.

Serial No. NDB(I)-66 O/OCH 1292(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Physical Chemistry of Nucleic Acids

Previous Serial Number: SAME

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: James A. Rose, M.D., Laboratory of Biology of Viruses, NIAID

This project has been discontinued.

Serial No. NDB(I)-67 O/OCH 1433(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Chemistry of the Vitreous Body

Previous Serial Number: SAME

Principal Investigator: Ralph J. Helmsen, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

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|---|---|
| Total: | 1.0 |
| Professional: | 1.0 |
| Other: | 0.0 |

Project Description:

Objectives: To determine physical and chemical parameters related to the molecular structure of vitreous macromolecules with the view of examining these for significance in the morphology of the vitreous body.

Methods Employed: Two major lines of investigation have been undertaken: isolation of a distinct protein from the vitreous of cattle and immunological examination of that protein to determine the tissue of its origin. Ammonium sulfate precipitation and column chromatography are used to fractionate and isolate the bovine vitreous protein. Analytical ultracentrifugation, intrinsic viscosity, optical rotatory dispersion, amino acid analysis and carbohydrate analysis are used for characterization. Immunological comparison between the vitreous protein and similar proteins derived from other tissues is effected through immunodiffusion and precipitation techniques as well as by immunoelectrophoresis.

The latter procedures will also be employed in part, to distinguish the isolated protein from paraproteins which may occur in the vitreous of animals suffering from certain eye abnormalities.

Major Findings: Soluble proteins and glycoproteins of the vitreous were separated from contaminating mucopolysaccharides on DEAE-Sephadex after preliminary dialysis against neutral 0.005 M phosphate buffer. The major ultraviolet absorbing peak obtained from chromatography was fractionated according to the procedure of Weimer and associates for serum orsomucoid. Material isolated as a precipitate with ammonium sulfate from the pH 3.7 filtrate was chromatographed on CM-Sephadex. After several step increases in pH a peak was eluted from the column in neutral 0.2 M phosphate and subsequently dialyzed against this buffer. Further purification of this material was effected on Sephadex G-25 in the same buffer. Ultracentrifugal analysis of the resulting solution by sedimentation equilibrium revealed a major component of approximately 16,000 (80%) and a minor component of approximately 90,000 (20%).

Significance to Bio-medical Research and the Program of the Institute: These projects represent continued study by the investigator into the interrelationship of the molecular structure of the macromolecular constituents of the vitreous body to the structure of this avascular connective tissue. It provides the base for determination of possible alterations in the tertiary structure of these macromolecules in various diseases states affecting the vitreous.

Proposed Course of Project: In addition to further characterization of the two components already discussed under Major Findings, new protein macromolecules will be isolated from the vitreous through modifications made in the chromatographic procedures presently being employed. Each distinct constituent thus obtained will be identified, if possible, by the immunological techniques referred to in the Methods Section. The ultimate goal of this systematic attack on the structure of the individual proteins of the vitreous is to furnish needed information in order to construct a working model to explain the gel-liquid structure of this connective tissue. The studies conducted on chickens with photo-induced enlargement of the vitreous and on buphthalmos will be resumed as soon as new animal facilities are available.

Honors and Awards: None

Publications:

Helmsen, R. J. and Mage, R. G.: Identification of immunoglobulin G in the vitreous of rabbits. Exp. Eye Res. 7: 121-128, Jan. 1968.

Serial No. NDB(I)-68 O/OCH 1530(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biological Oxidations in the Retinal Pigment Epithelium

Previous Serial Number: NONE

Principal Investigator: Hitoshi Shichi, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.2 |
| Other: | 0.0 |

Project Description:

Objectives: The retinal pigment epithelium has been shown histochemically to be of major significance in the metabolism of the retina and thus has a vital role in the visual process. The objective of this project is to investigate oxidative metabolism of the pigment epithelium with particular attention to the role of the endoplasmic reticulum in retinal-retinol interconversion and melanin formation.

Methods Employed: The endoplasmic reticulum of the retinal pigment epithelium was collected as microsomal particles by differential centrifugation of the tissue homogenate in sucrose-phosphate buffer. The oxidation of the reduced pyridine nucleotides (NADH and NADPH) by the microsomes was followed spectrophotometrically by a decrease in optical density at 340 mμ under various experimental conditions. A haemoprotein component in the microsomal electron transfer system was solubilized with trypsin and purified by fractionation with ammonium sulfate and by chromatography on Sephadex.

Major Findings: (1) Microsomes of the retinal pigment epithelium contained a CO-binding haemoprotein which had previously been found active in sterol hydroxylation and oxidative metabolism of drugs in various tissues. Cytochromse $b_5$ was not detected in the microsomes. (2) The haemoprotein could be reduced by pyridine nucleotides (NADH and NADPH), and the reduced

form was extremely autoxidizable; it could function as the terminal oxidase of the pigment epithelium microsomes. The haemoprotein could be solubilized by treatment of the microsomes with trypsin and purified on Sephadex. Spectral and structural properties of the purified protein were studied and evidence was obtained supporting its identity with a haemoprotein previously solubilized from heart microsomes. (3) The haemoprotein could be reduced with 3,4-dihydroxy phenylalanine which greatly stimulated the oxidation of the pyridine nucleotides.

Significance to Bio-medical Research and the Program of the Institute: The retinal pigment epithelium is known to possess a compact lattice of smooth surfaced endoplasmic reticulum, a part of which is apparently transformed into melanin granules. Melanin formation is generally believed to be catalyzed by a single enzyme, tyrosinase. However, our knowledge about this enzyme in the retinal pigment (and in animal tissues as a whole) is very limited and it is quite possible that this activity consists of a complex enzyme system. The microsomal electron transfer system investigated in the present work may function as such a complex enzyme system, since it is capable of rapid oxidation of pyridine nucleotides and reduction of the CO-binding haemoprotein if 3,4-dihydroxyphenylalanine (a possible intermediate in melanin formation) is present. In the presence of this compound, the oxidized forms of the pyridine nucleotides are increased which, in turn, will regulate the supply of retinal for rhodopsin synthesis by stimulating oxidation of retinol to retinal. Since it is very likely that the haemoprotein found in the pigment epithelium may also be involved in drug metabolism, as in other tissues, the microsomal electron transfer system studied here may have a clinical significance in the drug metabolism of the pigment epithelium.

Proposed Course of Project: (1) Solubilization and purification of other components in the microsomal electron transfer system and reconstitution of the system with well-defined components. (2) Metabolism of tyrosine and other intermediates for melanin synthesis by the microsomes and other cellular fractions, and definition of the individual enzymes and mechanisms of the reactions. (3) Physicochemical studies of the haemoprotein solubilized and purified from the retinal pigment epithelium.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 O/OM 1531(c)
1. Ophthalmology Branch
2. Section on Ophthalmic Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Synthesis of Sugar-Containing Polymers in Retina

Previous Serial Number: NDB(I)-66 O/CB 1294(c)

Principal Investigator: Paul J. O'Brien, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.5 |
| Other: | 0.2 |

Project Description:

Objectives: To determine the kinetic properties and co-factor requirements of a galactose transfer system present in bovine retina, which catalyzes the addition of galactose residues to pre-formed acceptor glycoproteins. To obtain unequivocal evidence for the sequential addition of galactose and N-acetyl neuraminic acid (NANA) to these acceptors. To determine some of the properties of an N-acetyl glucosamine transfer system and to identify the product as a glycoprotein. To obtain information regarding the location of these enzymes and the glycoprotein products in the retina.

Methods Employed: Bovine retinas are homogenized and the microsomal-mitochondrial fraction is isolated by differential centrifugation. The transfer of sugars to this particulate fraction is measured as acid insoluble radioactivity after incubation with appropriate radioactive sugar nucleotides. Products

are identified as glycoproteins by their sensitivity to proteolytic enzyme digestion and to glycosidase action. Whole retinas are incubated with radioactive sugars and the subcellular site of incorporation is determined by homogenization and differential centrifugation followed by counting in a scintillation spectrometer.

Major Findings: 1) Galactose is transferred from UDP-galactose to an endogenous acceptor with a Michaelis constant (Km) of 6.5μM for UDP-galactose. 2) This reaction shows an almost absolute requirement for $Mn^{++}$ ions with a maximum rate of transfer at 2.5 to 5 mM. 3) The amount of galactose incorporated by any given preparation is always equal to or slightly greater than the increment in NANA transfer caused by the addition of UDP-galactose to the NANA transfer system. 4) This stimulation of NANA transfer by UDP-galactose shows the same $Mn^{++}$ requirement as does galactose transfer. 5) Neuraminidase releases most of the NANA from ($^{3}H$) NANA-labeled peptides. However, a NANA-substituted ($^{14}C$) galactose-labeled peptide resists B-galactosidase treatment unless first treated with neuraminidase. The evidence indicates a sequential addition of galactose and NANA to the same carbohydrate chain of the acceptor. 6) N-acetyl glucosamine is also transferred from UDP-N-acetyl glucosamine to an endogenous acceptor and the product is digestible by proteolytic enzymes. 7) Preliminary experiments indicate that ($^{14}C$) glucosamine is rapidly incorporated by retina microsomes and that the rod outer segments become labeled in time.

Significance to Bio-medical Reserach and the Program of the Institute: The enzyme systems necessary for the biosynthesis of glycoproteins are present in the retina. These systems have been demonstrated in homogenates and are known to be functioning in whole retinas. The glycoprotein products appear to be structural components of the cell membranes, in particular the outer segments of the photoreceptors. Since these photoreceptors seem to be regenerated in a fairly short time (1-2 weeks in laboratory animals), defects in any of the enzymes involved in membrane synthesis might manifest themselves as progressive degeneration of the rods. Detailed knowledge of these processes will permit testing in model systems.

Proposed Course of Project: To examine the properties of transfer systems that add N-acetyl glucosamine and mannose to endogenous acceptors. To follow the time course of labeling

subcellular fractions during incubation of whole retinas with radioactive sugars. To study the effects of environmental conditions, light in particular, on these processes.

Honors and Awards: None

Publications:

O'Brien, P. J. and Muellenberg, C. G.: Incorporation of D -($^{14}$C) galactose and N -($^{3}$H) acetylneuraminic acid into glycoprotein by particles from bovine retina. Biochim. Biophys. Acta. (in press), 1968.

Serial No. NDB(I)-68 O/OM 1532(c)
1. Ophthalmology Branch
2. Section on Ophthalmic Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Aminosugar Metabolism in the Retina

Previous Serial Number: NDB(I)-66 O/CB 1295(c)

Principal Investigators: Paul J. O'Brien, Ph.D.
Roger G. Mazlen, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.8 |
| Professional: | 0.6 |
| Other: | 0.2 |

Project Description:

Objectives: To study the metabolism of aminosugars in the retina. To complete the study of the feedback inhibition of L-glutamine-D-fructose 6-phosphate amidotransferase isolated from bovine retina. To compare the characteristics of this enzyme with its counterpart in other tissues. To determine the role of this enzyme in the regulation of retinal glycoprotein and polysaccharide synthesis. To study the feedback inhibition of UDP-N acetyl glucosamine 2-epimerase and determine what regulatory function it may perform.

Methods Employed: Bovine retinas are homogenized and the enzyme is isolated by differential centrifugation, ammonium sulfate fractionation, and dialysis. The kinetic properties of the enzyme are studied by measuring the rate of product formation as a function of variable substrate concentrations. The effect of

inhibitors and inactivators is measured in the same way, using a specific colorimetric assay to determine the amount of product formed.

Major Findings: 1) The enzyme L-glutamine D-fructose 6-phosphate amidotransferase, which catalyzes the formation of glucosamine 6-phosphate exhibits a Michaelis constant (Km) of 0.35 mM for glutamine and 0.4 mM for fructose 6-phosphate. 2) The enzyme exhibits feedback inhibition by UDP-N-acetyl glucosamine, the end product of the pathway. 3) The inhibitor converts the enzyme to a form which is inhibitable by L-glutamine, one of the substrates, but not by fructose 6-phosphate, the other substrate. 4) The inhibitor constant (Ki) for UDP-N-acetyl glucosamine is 3 μM. 5) Maximum inhibition occurs at a glutamine concentration of 4 mM, the physiological level reported by Krebs. 6) An analog of L-glutamine, 6-diazo-5-oxo-L-norleucine (DON) exhibits competitive inhibition with respect to L-glutamine and also partially relieves the inhibition produced by UDP-N-acetyl glucosamine. 7) No detectable activity of UDP-N-acetyl glucosamine 2-epimerase could be found.

Significance to Bio-medical Research and the Program of the Institute: These results have described a previously unrecognized type of control mechanism whereby rising end product levels can alter a key enzyme in such a way that it can be inhibited by one of the substrates. The usual feedback inhibitory effect of rising end product levels is amplified by coupling with normally high substrate levels. The enzyme is thus brought under more sensitive and strict control.

The control of this key enzyme in retina is significantly different from that in other tissues, such as liver. In the latter, the end product exerts a direct competitive inhibition of the enzyme. In the former, advantage is taken of the high level of glutamine to bring the synthesis of UDP-N-acetyl glucosamine under very efficient control, thus narrowly limiting the range of biosynthetic rates of glycoproteins and mucopolysaccharides and making these processes more responsive to the immediate needs of retinal metabolism.

Proposed Course of Project: Completed

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 O/OM 1533(c)
1. Ophthalmology Branch
2. Section on Ophthalmic Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Protein Synthesis in the Retina

Previous Serial Number: NONE

Principal Investigators: Jacqueline J. Bungenberg de Jong, Ph.D.
Paul J. O'Brien, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.1 |
| Professional: | 0.9 |
| Other: | 0.2 |

Project Description:

Objectives: To study the characteristics of protein synthesis in the retina. To determine the optimal conditions for in vitro incorporation of radioactive amino acids into protein of bovine retina incubated as a tissue slice. To determine whether such protein synthetic activity follows the now generally accepted pathways with regards to incubation conditions and known inhibitors of protein synthesis.

Methods Employed: Bovine retinas, isolated in such a fashion that as little damage as possible is done to the tissue and that contamination with pigment epithelium is kept to a minimum, are incubated in Krebs-Ringers isotonic buffer in the presence of uniformly labeled $^{14}C$-leucine. Incorporation of the precursor amino acid into protein is studied under varying conditions such as after the addition of nutrients and metabolic inhibitors.

Streptomycin and penicillin are added to the incubation medium to prevent bacterial contamination. Incorporation of precursor into protein is measured as acid precipitable, lipid solvent insoluble radioactivity detected in a scintillation counter. The compartmentalization of the radioactivity within the retina is studied by isolation of subcellular fractions by differential centrifugation. The nature of the radioactive proteins is studied employing starch electrophoresis and polyacrylamide electrophoretic techniques.

Major Findings: 1) Uptake of $^{14}C$-leucine into the protein of the bovine retina is extremely dependent on sufficient aeration of the medium and requires the presence in the medium of an oxidizable substrate (0.02 M glucose) and very small amounts of all 22 amino acids (0.1 mg casein hydrolysate /10 ml medium). 2) Under optimal conditions continuous incorporation into total protein can be observed for as long as six hours. 3) The microsomal fraction, which initially has the highest specific activity (cpm/mg protein), reaches a plateau after 60-90 minutes, but the specific activity of the mitochondrial and rod fractions continues to rise. 4) The presence of $10^{-3}M$ puromycin inhibits incorporation of $^{14}C$-leucine into protein by 85-90% in one hour. 5) Protein synthesis by dark adapted retinas in the dark is more active than by bleached retinas (50% more radioactivity incorporated after three hours in the dark than in the light).

Significance to Bio-medical Research and the Program of the Institute: The study of the synthesis of the apoprotein of rhodopsin may contribute considerably to the knowledge and understanding of metabolic defects in rhodopsin biosynthesis and possibly explain some forms of functional impairment of the retina.

Proposed Course of Project: To study and identify the products of protein synthesis by beef retina *in vitro*, with emphasis on the proteins of the rod outer segments in order to determine whether rhodopsin or its apoprotein can be synthesized by the retina under dark adpated and/or bleached conditions.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-56 O/OPH 301(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Study on the Pharmacodynamics of Various Agents Affecting the Intraocular Pressure

**Previous Serial Number:** SAME

**Principal Investigator:** Frank J. Macri, Ph.D.

**Other Investigators:** None

**Cooperating Units:** None

**Man Years**

| | |
|---|---|
| Total: | 0.95 |
| Professional: | 0.45 |
| Others: | 0.5 |

**Project Description:**

Objectives: To determine the pharmacodynamics of agents able to alter the intraocular pressure with a view toward finding more effective compounds and to possibly further the understanding of mechanisms which maintain the intraocular pressure.

Methods Employed: All agents investigated were examined on the cat eye. The preparations used were: 1) The intact living eye. 2) The arterially perfused enucleated eye. 3) The arterially perfused, isolated iris-ciliary body and iris preparations. The various functions measured were the intraocular pressure, iris artery pressure, and perfusate flow rate. Constant rate perfusion of the anterior chamber was used to measure the rate of aqueous humor formation.

Major Findings: Eserine, eserine + acetylcholine, acetylcholine, l-epinephrine, l-norepinephrine, isoproterenol, tolazoline + l-epinephrine, hexamethonium, sodium nitrite, pitressin, ergotamine and guanethidine were studied for their effects on the rate of aqueous humor inflow. With the exception of hexamethonium, the autonomic agents of this series either had no effect or increased the rate of aqueous humor formation; hexamethonium and sodium nitrite decreased the rate. Pitressin and ergotamine had no measurable activity.

Significance to Bio-medical Research and the Program of the Institute: Eserine, eserine + acetylcholine, l-epinephrine and isoproterenol were found to increase the rate of aqueous humor inflow. This is unexpected since many investigators have found these agents effective in lowering eye pressure in animals and in human subjects; l-epinephrine has been reported to actually decrease inflow. The inhibitory activity of sodium nitrite and hexamethonium is probably due to its blood pressure lowering response.

Proposed Course of Project: The activity of these agents will be restudied using a scintillation probe over the cornea to determine the decay rate of intracamerally administered $^{125}I$ RISA. In this attempt of a confirmatory study, steady-state conditions cannot be altered as is possible with the present technique. In addition it is intended to delve more deeply into the role of blood pressure on formation rate. Present indications are that ultrafiltration may play a greater role in the elaboration of aqueous humor than had heretofore been recognized.

Honors and Awards: None

Publications:

Macri, F. J.: The pressure dependence of aqueous humor formation. Arch. Ophthal. 78: 629-633, Nov. 1967.

Serial No. NDB(I)-59 O/OPH 600(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Role of Vasculature in the Maintenance of Intraocular Pressure

Previous Serial Number: SAME

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.95 |
| Professional: | 0.45 |
| Other: | 0.5 |

Project Description:

Objectives: To determine if and by what mechanisms the dynamics of the intraocular vasculature affect the eye pressure and to demonstrate the pharmacologic response of the vasculature to certain drugs.

Methods Employed: The enucleated cat eye or either of its two major components, the anterior or posterior segment, was perfused under constant pressure through the ophthalmic or long posterior ciliary artery. Changes in the measured perfusate flow rate indicated the type of vascular response obtained, i.e. vasoconstriction or vasodilation. The site of drug action could be ascertained by surgical removal of various parts of the preparation and subsequent re-evaluation of drug activity.

Major Findings: Considerable effort has been expended in elucidating the mechanism of action of diverse pharmacologic agents which have a common vasoconstrictive action on the iris artery of the cat eye. Although this work is still incomplete, two interesting findings have been made: 1) The action of the B-adrenergic stimulating agent, isoproterenol, could be inhibited by either an a-adrenergic blocking agent (tolazoline) or by a p-sympathominetic blocking agent (atropine); and 2) The actions of ouabain, eserine, ergotamine, epinephrine, norepinephrine, isoproterenol and acetazolamide could all be inhibited by the B-blocking agents pronethalol or propranolol. Further studies have shown that the blockade of the actions of these agents may not be related to the B-adrenergic system since now we also find that d-propranolol (which is an extremely weak blocker) also has marked inhibitory actions on the vasoconstrictor response.

Significance to Bio-medical Research and the Program of the Institute: These findings are of marked interest from two points of view: 1) They demonstrate a unique reactivity of the iris artery; and 2) They suggest the use of d-propranolol in patients being treated for glaucoma to indicate the extent by which therapeutic agents act on the vasculature of the eye.

Proposed Course of Project: It is planned to continue this project along the same lines as for the current year. All of the autonomic nervous system drugs will be studied and the pharmacodynamics of the active agents clarified.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 O/OPH 1434(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: A Study of the Buphthalmic Rabbit Eye

Previous Serial Number: SAME

Principal Investigators: Frank J. Macri, Ph.D.
P.R.B. McMaster, M.D., LGAR, NIAID

Other Investigators: None

Cooperating Units: Laboratory of Germfree Animal Research, NIAID

Man Years

| | |
|---|---|
| Total: | 0.4 |
| Professional: | 0.4 |
| Other: | 0.0 |

Project Description:

Objectives: Buphthalmia in rabbits has generally been considered as the animal equivalent of the human disease, infantile glaucoma. It was felt that observations on the buphthalmic rabbit eye might shed some light on other abnormalities of eye pressure regulation.

Methods Employed: Elasticity of the coats of the eye were determined by injecting a fixed volume of fluid into the anterior chamber and recording the eye pressure change. The determinations were made over the eye pressure range of 15 mm Hg to 60 mm Hg. The data of each rabbit was expressed in a plot of IOP( mean)= $\frac{\Delta P}{\Delta V}$, where $\Delta P$ is the incremental pressure produced by the injection of a fixed volume ($\Delta V$) of fluid. The slope of this function was

then plotted against the aqueous humor volume for each eye. The aqueous humor volume was considered as being representative of the degree of buphthalmia of the eye.

Major Findings: Approximately twenty buphthalmic eyes have been investigated. With a single exception, the elasticities of these eyes have been found to be greater than those obtained with normal eyes.

Significance to Bio-medical Research and the Program of the Institute: Current indications from this research suggest that rabbit eyes become buphthalmic because of an inability of the coats of the eye to withstand the stretching force of the intraocular pressure.

Proposed Course of Project: It is intended to determine the elasticity of rabbit eyes during the interval from weaning to maturity. In this manner we can more accurately conclude whether the elastic changes are a cause or a result of buphthalmia.

Honors and Awards: None

Publications:

McMaster, P.R.B., and Macri, F.J.: Secondary aqueous humor outflow pathways in the rabbit, cat and monkey. Arch. Ophthal. 79: 297-303, March 1968.

Serial No. NDB(I)-63 O/OC 1022(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Methotrexate Therapy of Selected Patients with Uveitis

Previous Serial Number: SAME

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.3 |
| Professional: | 0.3 |
| Other: | 0.0 |

Project Description:

Objectives: This project is being continued to explore the role of antimetabolites in the treatment of refractory ocular inflammatory conditions.

Methods Employed:

I. Drug Therapy

1) Methotrexate is given intravenously twice weekly at 25 mgm/$M^2$ body surface area.
2) Cyclophosphamide is administered weekly at 1 gm/$M^2$ body surface area.

II. Laboratory Studies

1) Standard radial immuno-diffusion technique was used to

quantitate the three classes of immunoglobulins (IgG, IgA, IgM) in the sera of treated patients. The levels of immunoglobulins were compared prior to and after cessation of immunosuppressive therapy.

2) Primary immune response was examined by administering subcutaneously 0.1 mgm of Vi (Vi), tularemia vaccine (Tu), and pneumococcus (Pn) antigens. Antigen was given one week after the start of therapy. Antibody titres were determined before and at two and four weeks after treatment had begun.

3) Blood for determination of iso- and febrile agglutinins was obtained to ascertain the effect of immunosuppressive therapy on secondary antibody formation.

4) After immunosuppression had begun, a sensitizing dose of 2,000 ug DNCB (2, 4, Dinitrochlorobenzene) was applied to the skin of the upper arm. Testing doses of 50 ug and 100 ug were reapplied at two weeks. This is performed to evaluate the influence on the induction of delayed hypersensitivity.

5) Positive Mantoux tests obtained on admission was considered as evidence of delayed hypersensitivity. Patients with positive reactions were rechallenged with the same antigen during the on-therapy period four to six weeks after chemotherapy had begun.

6) The effect of systemically used antimetabolites on an induced local inflammatory response was studied by employing the skin-window technique. Each patient served as his own pretreatment control. The procedure was repeated after two weeks of therapy and 24 hours after the injection of chemotherapy.

Major Findings: To date 30 patients have been treated with immunosuppressive agents. Clinical improvement ranged from complete remission of signs of inflammation to partial remission which was more easily controlled with corticosteroids. Twenty-five patients had active uveitis and 19 responded favorably to therapy. Two patients who had failed to respond on Methotrexate did so on Cytoxan. Five patients whose sympathetic ophthalmia was controlled with steroids had surgery performed on the remaining eye with the addition of immunosuppressive therapy. Good surgical results were obtained in each case. Three patients with Behcet's syndrome, one with unilateral iridocyclitis, and one with uveal effusion did not improve on treatment.

Marked suppression of primary antibody formation to antigens was seen in 13 patients who were tested during the on-therapy period. Complete suppression was seen in eight. No significant

depression of secondary antibody levels was found in any of the 19 patients tested following chemotherapy. Similarly no significant alterations in response to chemotherapy were discovered within each class of immunoglobulins (IgG, IgA, and IgM). With respect to delayed hypersensitization, complete suppression was found in four of six patients stimulated with DNCB during the treatment period. The suppression of established delayed hypersensitivity occurred in three of nine patients, modified reaction occurred in two, and no effect was observed in four.

Varying degrees of altered immune responses were obtained with antimetabolites but consistent correlation between clinical results and the extent of immunosuppression could not be established. However, the effectiveness of these agents may be inherent with the anti-inflammatory properties of these agents. From the skin-window studies on 13 patients, significant alteration of inflammation was observed in each case undergoing treatment. The median control values for percentage of mononuclear cells in the skin-window were 3, 22, and 86% for the 4, 8, and 24 hour stages of inflammation. The effect of chemotherapy for the same interval showed a reduction to 1, 14, and 37 median percentage of mononuclear cells.

The effectiveness of short-term (treatment during hospitalization) immunosuppressive therapy in refractory ocular inflammation has been encouraging. However, long-term administration of these agents must be tried before their application could be recommended.

Significance to Bio-medical Research and the Program of the Institute: This project is combined within the Branch's program on uveitis.

Proposed Course of Project: The project is being continued.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 O/OC 1435(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Ocular Complications of Vincristine (Oncovorin)

Previous Serial Number: SAME

Principal Investigators: Daniel M. Albert, M.D.
Vernon G. Wong, M.D.

Other Investigators: Edward Henderson, M.D.,
Medicine Branch, NCI

Publications:

Albert, D. M., Wong, V. G., and Henderson, E. S.: Ocular complications of vincristine therapy. Arch. Ophthal. 78: 709-713, Dec. 1967.

This project is complete.

Serial No. NDB(I)-67 O/OC 1436(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Toxic Effects of Drugs on the Retina - Experimental Studies on Chloroquine and Iodoacetate

Previous Serial Number: SAME

Principal Investigators: Eliot Berson, M.D.
Peter Gouras, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

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|---|---|
| Total: | 0.8 |
| Professional: | 0.4 |
| Other: | 0.4 |

Project Description:

Objectives: To study and compare the effect of chloroquine and iodoacetate on retinal function with correlation of electrophysiological changes, ophthalmoscopic findings, and histopathology.

Methods Employed: Electroretinograms are recorded from both eyes simultaneously in the anesthetized cat, before one eye is subjected to local drug injections. Electroretinography is used to measure the effects of drugs on retinal function, and the eyes are studied ophthalmoscopically and histologically at varying intervals after injection. Drugs are injected by the intravitreal route or through the long posterior ciliary artery.

Major Findings: Experiments show that electroretinograms recorded from both eyes of the same animal simultaneously are

symmetrical. With intravitreal injection of iodoacetate or chloroquine, depression of both major components of the electroretinograms has been observed. The uninjected eye in each animal has remained normal. The threshold dose of chloroquine and iodoacetate to produce damage to the receptor cell layer has been determined and the effects of equimolar concentrations of the drugs have been compared.

Injection of equimolar concentrations of iodoacetate or chloroquine into the long posterior ciliary artery has also been done and the histology of these eyes are under review. Electrophysiology reveals acute toxicity with iodoacetate by this route but no toxicity with chloroquine in similar concentrations.

Significance to Bio-medical Research and the Program of the Institute: Chloroquine has had widespread use as an antimalarial and in the treatment of arthritis. The retinal toxicity observed clinically is not well understood. An experimental model is needed to study its effects on retinal function and to explore the possibility of pharmacologic antagonism. Through the study of retinal toxicity more may be learned about the physiology of the nervous system.

Iodoacetate produces a known retinal toxicity when given by the systemic vascular route and has been used as a control in this model for comparison with the findings with chloroquine. Such comparison of intravitreal and long posterior ciliary artery injections may help to clarify the role of the blood brain-barrier in these toxicities.

Proposed Course of Project: Studies are in progress to define the retinal toxicity of chloroquine and iodoacetate following injection of these drugs in the vitreous or in the long posterior ciliary artery correlating electroretinography, ophthalmoscopy, and histopathology. Various doses of drugs have been tried in the cat. When the experimental model is well defined, pharmacologic antagonism studies can be considered.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 O/OC 1437(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

**PHS-NIH**
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Immunoglobulins in Uveitis

Previous Serial Number: SAME

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: None

This project has been incorporated with NDB(I)-63 O/OC 1022(c)

Serial No. NDB(I)-68 O/OC 1534(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Retinal and Uveal Changes Produced in Monkeys by Inoculation of Heterologous CNS Antigen and Homologous and Heterologous Retinal Antigen

Previous Serial Number: NDB(I)-67 O/CH 1419(c)

Principal Investigators: Ludwig von Sallmann, M.D.
Ronald Myers, M.D., Ph.D.
Edwin Lerner, M.D.
Stanford Stone, D.Sc.

Other Investigators: None

Cooperating Units: Laboratory of Perinatal Physiology, NINDB (Puerto Rico) NDB(I)-65 LPP 1263
Laboratory of Immunology, NIAID

Man Years

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|---|---|
| Total: | 0.9 |
| Professional: | 0.3 |
| Other: | 0.6 |

Project Description:

Objectives: 1) To repeat experiments of inoculation of guinea pig spinal cord in monkeys for possible clarification of pathogenetic factors associated with the vasculo-occlusive retinopathy described in last year's report. 2) To analyze the ocular allergic responses in monkeys to inoculation of homologous and heterologous retinal antigen and tissues rich in basement membranes.

Methods Employed: Inoculation of guinea pig spinal cord antigen followed the procedure employed previously. The eyes of

experimental animals of this series were removed with particular care so that long portions of the optic nerve were preserved for histological examination. Additional animals received injections of monkey or bovine retina (100 mg per animal) emulsified in Freund's adjuvant. Homologous choroid plexus (30mg per animal) and guinea pig kidney antigens were prepared and administered in the same fashion.

Major Findings: Following inoculation with guinea pig spinal cord, a heavy mantle of granulomatous inflammation is frequently found surrounding the axial vessels in the optic nerve. In two eyes, an occlusion of the central artery of the optic nerve was demonstrated as well as congestion with hemostasis in the central vein. The disturbance of the circulation in these vessels may set the stage for the development of the hemorrhagic retinopathy.

Inoculation of homologous retinal antigen in ten monkeys produced in all eyes signs of "delayed allergy" in the uvea and/or retina without accompanying neurological deficits. The injection of heterologous retina and the other antigens were ineffective.

Involvement of the choroid and retina varied greatly. When the uveitis was severe and extensive (in three animals), it could not be decided whether the retinal vasculitis was a primary autoimmune response or was secondary to the inflammation in the choroid. In seven animals, however, the lesions in the uvea were focal, minimal, or even absent, and in these eyes the periphlebitis was considered to be a direct autoimmune lesion. The incipient change in the veins was the formation of a broad perivascular space probably as the result of altered vascular permeability. Round cells, predominantly lymphocytes, invaded this space and unwrapped the veins as cuffs or sheaths. The perivascular infiltrate sometimes extended into the inner granular layer but the outer strata of the retina retained their normal architecture. In later stages the perivascular infiltrate brought about a collapse of the afflicted vessels without creating hemorrhages. The inflammatory cells filled the previous site of the vein.

Significance to Bio-medical Research and the Program of the Institute: Autoimmune mechanisms might be operative in the development of intraocular inflammatory diseases. The resemblance of the experimentally induced autoimmune retinopathies to vascular retinopathies in man may suggest therapeutic approaches in the future.

Proposed Course of Project: If the cooperation of the three Units can be reactivated, the results of increasing the number of experiments and modifying the experimental approach would appear to be extremely promising.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 O/OC 1535(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies of Cellular Proliferation in Ocular Tissues

Previous Serial Number: NDB(I)-67 O/CH 1432(c)

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia Grimes, B.A.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.9 |
| Professional: | 1.2 |
| Other: | 0.7 |

Project Description:

Objectives: 1) To continue investigation of the cause of the marked stimulation of mitotic activity in the lens epithelium during the early phase of sugar cataract development. 2) To establish conditions for maintenance of the rat lens in vitro such that cell division and differentiation in the epithelial layer remain sufficiently normal to study the effects of various experimental interventions.

Methods Employed: Cataracts were induced in galactose or xylose-fed and alloxan diabetic rats and mitotic activity of the lens epithelium was studied by methods reported previously.

Rat lenses were maintained in vitro in modified Merriam-Kinsey culture tubes. The basic medium employed was a mixture of TC 199 and bicarbonate buffer in equilibrium with a gas phase

of 95% air and 5% $CO_2$. All lenses were examined under magnification when they were removed from the culture tubes to determine if opacities were present; they were then fixed in ethanol-acetic acid and flat mounts of the epithelium were prepared.

Major Findings: 1) Sugar cataracts. A spurt of cell proliferation in the lens epithelium of galactose-fed rats occurs in the absence of morphological signs of cell injury or disorganization of the epithelial layer and the increase of mitotic activity shows a close temporal relation to lens swelling. These observations suggested that expansion of the epithelial area accompanying the increase of lens volume could be the stimulus for cell division. Further investigation of lenses of alloxan diabetic rats, however, failed to support this hypothesis. In these animals the lens epithelium shows a burst of proliferative activity similar in time course to that induced by galactose though of less magnitude, but in the absence of any detectable lens swelling.

To examine more closely the relation between lens swelling and mitotic stimulation in galactose cataract we maintained rat lenses in vitro under such conditions that the osmotic swelling which takes place in the presence of high levels of galactose could be controlled by appropriate variations in the tonicity of the medium. Lenses incubated for 24 hours in a medium containing 30 mM galactose developed typical early galactose cataracts, but the lens epithelium was frequently damaged. Cells were overlapping, pyknosis or nuclear swelling were common, and mitotic activity was severely suppressed. The toxic effect of galactose in vitro prevented the appearance of the proliferative response and the possibility of testing its relation to lens swelling. However, when the tonicity of the bathing medium was gradually increased during the incubation period, all of the damaging effects of galactose could be prevented, demonstrating that they are due to an osmotic imbalance of the epithelial cells.

2) Studies of the epithelium of normal lenses maintained in vitro. Lenses incubated in the basic medium demonstrated an abrupt fall in mitotic activity to approximately 10% of normal values within the first two hours of culture, but a more normal rate of cell division was resumed by four hours. At 24 hours mitotic activity was 70% of normal. The number of cells synthesizing DNA decreased slowly to 50% of normal at the end of 24

hours. The cells of the proliferating epithelium showed no abnormalities during this interval. An unexpected observation was the appearance of nuclear fragmentation and cell death among the differentiating cells of the meridional rows which began by ten hours and was very marked at 24 hours. After two days of culture when mitotic activity is reduced to 25% of normal the meridional rows are disorganized and contain relatively few cells.

The addition of small quantities of either bovine or rat serum (1%) or insulin (0.5 ug/ml) prevents the occurrence of nuclear fragmentation in the rows. However, both serum and insulin ultimately induce a disorganization of the epithelial layer and an abnormal wave of mitotic activity. Even under these conditions, when direct destruction of differentiating cells is prevented, the population of the rows is reduced after 48 hours suggesting an impairment of the flow of cells from the proliferating compartment to the differentiating compartment.

Significance to Bio-medical Research and the Program of the Institute: The lens epithelium is a relatively simple cell system particularly well-suited to study the life cycle of the cell. Knowledge of the population dynamics which is obtained in this tissue under physiological and pathologic conditions can be related to the problem of cellular proliferation and its abnormalities in general.

Proposed Course of Project: Efforts will be continued to define the conditions under which the lens can be maintained in some degree of normality with regard to proliferation and differentiation of the epithelial cells. The role of other hormones known to be effective in other organ culture systems will be examined.

Honors and Awards: None

Publications:

Grimes, P. and von Sallmann, L.: Lens epithelium in sugar cataract. Invest. Ophthal. (in press), 1968.

Serial No. NDB(I)-68 O/OC 1536(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Evaluation of the Ocular Hazards of Transscleral Laser Radiation

Previous Serial Number: NDB(I)-67 O/CH 1431(c)

Principal Investigators: Richard S. Smith, M.D.
Marvin N. Stein, Ph.D.

Other Investigators: None

Cooperating Units: Biophysics Branch, Armed Forces Institute of Pathology

Man Years

| | |
|---|---|
| Total: | 0.30 |
| Professional: | 0.10 |
| Other: | 0.20 |

Project Description:

Objectives: To measure the physical characteristics of the sclera, choroid, and retina in relation to the transmission of light through these layers and to evaluate the ocular effects of transscleral laser radiation in living rabbits.

Methods Employed: The spectral reflection and transmission of light incident upon the sclera is measured using a Beckman DKIA spectrophotometer with an attached DKA spectroreflectometer. Results obtained by this technique are checked by an alternate method in which lasers act as the source of radiant energy and energy transmission is measured by a cone calorimeter. Pigmented rabbit eyes and human autopsy eyes are studied by both techniques. The eyes of adult pigmented rabbits are exposed to laser radiation via the sclera. After an interval of five days, the animals are

sacrificed and their eyes are subjected to routine histological techniques.

Major Findings: To date, the spectral reflection and transmission characteristics of human and pigmented rabbit sclera, choroid, and retina have been measured by the spectrophotometer technique. The spectral range covered extends from 500-2600 nanometers. The qualitative pattern of the curves obtained is similar in human and rabbit eyes. The rabbit eyes show a transmission about 10% greater than the human eyes at all wavelengths. Two laser sources are available to use, operating at 694.3 and 1060 nanometers. The transmission of light at these two wavelengths was measured with the laser/calorimeter system. Results with this technique gave a value for transmission of about 5% less than those obtained by the spectrophotometer technique. A total of 70 living rabbits have been irradiated by the ruby and neodymium lasers. The thresholds for transscleral injury are approximately 30 joules/$cm^2$ for the ruby laser and 50-60 joules/$cm^2$ for the neodymium laser. The lesions produced are similar for both lasers. These include: production of posterior subcapsular cataracts, corneal vascularization, intraocular hemorrhage, retrocorneal membrane formation, and chorioretinal-vitreal lesions varying in size dependent upon the intensity of the laser beam.

Significance to Bio-medical Research and the Program of the Institute: The widespread use of lasers in clinical ophthalmology and for industrial and scientific applications results in the exposure of many workers to radiation from these devices. Assessments of the possible ocular effects of intentional and accidental exposures of the eye depends on an understanding of the amount of light transmitted at different wavelengths. Additional information is provided by in vivo experiments.

Proposed Course of Project: This project is completed and the results of the in vivo experiments are being prepared for publication.

Honors and Awards: None

Publications:

Smith, R. S. and Stein, M. N.: Ocular effects of transscleral laser radiation I: spectral reflection and transmission of human and rabbit sclera, choroid, and retina. Amer. J. Ophthal. (in press), 1968.

Serial No. NDB(I)-68 O/OC 1537(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effects of Intrauterine Surgical Procedures on the Development of the Primate Eye

Previous Serial Number: NONE

Principal Investigators: Ludwig von Sallmann, M.D.
Ronald Myers, M.D., Ph.D.

Other Investigators: None

Cooperating Units: Laboratory of Perinatal Physiology, NINDB (Puerto Rico)

Man Years

| | |
|---|---|
| Total: | 0.3 |
| Professional: | 0.1 |
| Other: | 0.2 |

Project Description:

Objectives: To study the ocular abnormalities of neonatal rhesus monkeys that had been subjected to either bilateral ligation of the carotid arteries and jugular veins or transient compression of the umbilical cord at the middle of the gestation period. The surgical interventions were designed and carried out by Dr. Ronald Myers of the Laboratory of Perinatal Physiology in his studies of brain damage resulting from hypoxia during fetal life.

Methods Employed: The surgical technique is described elsewhere. The animals were delivered by cesarean section, as a rule and were killed after a short interval. Fixation was accomplished by perfusion from the heart with 10% formaldehyde solution. The eyes were then enucleated and placed in the same fixative. After removal of a superior calotte the globes were examined under the

dissecting microscope, embedded in paraffin, serially sectioned and stained.

Major Findings: Nine eyes from five neonatal monkeys were examined, and of these, seven showed abnormalities that resembled certain congenital deformities of the human eye.

Extensive cystic degeneration of the retina leading to retinoschisis was observed in both eyes of three monkeys. Two of the affected animals had been subjected to ligation of the carotid and jugular vessels and one to compression of the umbilical cord. The severity and dimensions of the lesions differed from one eye to another but the essential features were common to all. Splitting occurred in the plane of the nerve fiber layer as a result of the confluence of small cysts and was localized in the temporal midperiphery of the retina. The lesions bore a striking resemblance to the infantile form of retinoschisis in man. Both eyes of two monkeys which had cystic degeneration also demonstrated circumscribed foci of retinal destruction. The retina adjacent to the areas of atrophy was not involved in the process of cystic degeneration; therefore, a relation between these two types of lesions could not be established.

Leukomata were seen in the paracentral cornea of the left eyes of two infant monkeys. In both instances Descemet's membrane and endothelium were defective and persistent remnants of the pupillary membrane were attached to the rim of the corneal lesions. Circumscribed anterior polar cataract complicated by the adherence of a dense portion of the persistent pupillary membrane was present in a third animal and closely resembled congenital anterior polar cataracts of man. It is possible that the experimentally induced hypoxia at mid-term delayed the involution or produced shrinkage of the pupillary membrane affecting the structure of the anterior pole of the lens.

Significance to Bio-medical Research and the Program of the Institute: The demonstration of factors which produce teratological lesions in the eyes of primates is of importance for understanding and possibly preventing certain congenital abnormalities of man.

Proposed Course of Project: It is hoped that as more experimental material becomes available, data will be accumulated which will permit correlation of the eye changes with neurological changes and the circulatory alterations induced.

Honors and Awards: Invited to present a paper on findings:

Symposium on Embryonic Development, Association for Research in Ophthalmology National Meeting, October 1967.

Publications:

von Sallmann, L.: The effect of intrauterine surgical procedures on the development of the primate eye. Invest. Ophthal. (in press), 1968.

Serial No. NDB(I)-68 O/OC 1538(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: In Vitro Malignant Transformation of Hamster Retina, Choroid, and Iris with DNA Viruses

Previous Serial Number: NONE

Principal Investigators: Daniel M. Albert, M.D.
Alan S. Rabson, M.D.
Albert J. Dalton, Ph.D.

Other Investigators: None

Cooperating Units: Pathologic Anatomy Branch, NCI
Viral Studies, NCI

Man Years

| | |
|---|---|
| Total: | 0.25 |
| Professional: | 0.25 |
| Other: | 0.00 |

Project Description:

Objectives: To study abilities of SV40, LLE46, adenovirus 12, and polyoma virus to transform hamster retina and uveal tissues.

Methods Employed: Ocular tissues are removed from hamsters and grown in vitro. Some cultures are infected with virus and studied for changes in growth rate and pattern. After several passage $1 \times 10^8$ cells from each culture are injected subcutaneously. Resulting tumors are studied by light and electron microscopy.

Major Findings: Most cultures underwent transformation as evidenced by sustained rapid growth, development of an acid pH soon after instillation of fresh media, and formation of multiple layers of cells and clumps. After several in vitro passages,

transformed cells injected subcutaneously into irradiated adult hamsters produced tumors at the injection sites.

All ocular tissue transformed with SV40 and produced neoplasms composed of epithelioid and spindle-shaped cells with large nuclei; however, no pigmented tumors were observed. Choroid and iris transformed with LLE46 produced tumor histologically similar to the SV40 type growths. Retina infected with LLE46 produced tumors which were bimorphic, containing in addition to the SV40-like component, areas of small hyperchromatic cells resembling an adenovirus-induced tumor. Polyoma virus transformed only retina, producing tumors with elongated cells with hyperchromatic pleomorphic nuclei and small amounts of basophilic cytoplasm. Adenovirus 12 also transformed only retina and gave rise to typical adenovirus-type neoplasms composed of layers of small, darkly-staining cells. Although these tumors had some resemblance to retinoblastomas, no rosettes or neural elements could be identified.

Significance to Bio-medical Research and the Program of the Institute: These experiments are concerned with the possible role of virus in the development of intraocular malignancy and, therefore, have a direct relationship to the ocular tumor project.

Proposed Course of Project: Completion of the study by the end of this fiscal year is anticipated.

Honors and Awards:

A summary of this study was accepted for presentation at the Spring Meeting of the Association for Research in Ophthalmology, 1968.

Publications:

Albert, D. M., Rabson, A. M., and Dalton, A. J.: In vitro neoplastic transformation of uveal and retinal tissue by oncongenic DNA viruses. Invest. Ophthal. (in press), 1968.

Serial No. NDB(I)-68 O/OC 1539(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Episcleral Venous Pressure and Glaucoma

**Previous Serial Number:** NONE

**Principal Investigators:** Thomas F. Minas, M.D.
Steven M. Podos, M.D.
Frank J. Macri, Ph.D.

**Other Investigators:** Ralph D. Gunkel, O.D.

**Cooperating Units:** None

**Man Years**

| | |
|---|---|
| Total: | 0.6 |
| Professional: | 0.6 |
| Other: | 0.0 |

**Project Description:**

Objectives: To investigate the episcleral venous pressure in normal human eyes, eyes with primary open angle glaucoma, and eyes with other forms of glaucoma.

Methods Employed: The episcleral venous pressure was measured with a new instrument developed for this purpose. A modified force-displacement transducer with a lucite cone applanating tip was mounted on a slit lamp biomicroscope and applied to the desired vessel, one could then determine the pressure necessary to arrest blood flow. Measurements were made on a variety of human eyes, normal and glaucomatous.

Major Findings: The mean episcleral venous pressure of 39 normal eyes was 9.0 $\mp$ 1.4 mm Hg (standard deviation) and of 27 eyes with primary open angle glaucoma was 8.6 $\mp$ 1.4 mm Hg. There was no significant difference between these values.

A family was studied in which two members had glaucoma and associated idiopathic elevated episcleral venous pressure. The mother was affected bilaterally and her daughter in the right eye with distended episcleral veins, elevated intraocular pressures, glaucomatous field loss and disc changes, and mean episcleral venous pressures of daughter O.D. 19.1 $\mp$ 0.9 (standard error), O.S. 11.0 $\mp$ 0.8 and mother O.D. 18.2 $\mp$ 0.8, O.S. 17.9 $\mp$ 1.0 mm Hg. Cannulation of an episcleral vein of the right eye of the mother was carried out with direct measurement of the episcleral venous pressure. The results agreed favorably with external measurement.

Significance to Bio-medical Research and the Program of the Institute: The measurement of episcleral venous pressure is of importance in further understanding human aqueous humor dynamics. The diagnosis and therapy of some forms of glaucoma related to episcleral venous pressure elevation may be facilitated by elucidation of their pathogenesis.

Proposed Course of Project: The episcleral venous pressure of eyes with a variety of ocular diseases is being studied. Furthermore, this instrument will be evaluated regarding its usefulness in measuring the arterial episcleral pressure. It may be possible to estimate the ophthalmic artery pressure on the basis of the readings obtained on episcleral arteries.

Honors and Awards: None

Publications:

Podos, S. M., Minas, T. F., and Macri, F. J.: A new instrument to measure episcleral venous pressure: comparison of normal eyes and eyes with primary open angle glaucoma. Arch. Ophthal. (in press), 1968.

Minas, T. F. and Podos, S. M.: Familial glaucoma associated with elevated episcleral venous pressure. Arch. Ophthal. (in press), 1968.

Serial No. NDB(I)-68 O/OC 1540(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Histologic and Crystallographic Examination of Crystals in Eye Tissues of Patients Affected with Cystinosis

Previous Serial Number: NONE

Principal Investigators: Vernon G. Wong, M.D.
Paul D. Frazier, D.D.S.

Other Investigators: None

Cooperating Units: Laboratory of Histology and Pathology, NIDR

Man Years

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.2 |
| Other: | 0.0 |

Project Description:

Objectives: To determine the identity of crystalline deposits in ocular tissues of patients affected with childhood and adult cystinosis.

Methods Employed: 1) Eyes were obtained at autopsy from two patients with childhood cystinosis. 2) Conjunctival biopsies were obtained from two adult cases of cystinosis and from one of them a corneal lamellar button was removed by trephination for examination. The tissues were fixed in absolute ethyl alcohol and divided for routine histologic examination, polarization microscopy and x-ray diffraction. Tissues used for microscopy were embedded in paraffin and celloidin. 3) X-ray diffraction studies were carried out on those tissues containing crystalline

material and were mounted on the end of a glass capillary. 4) Diffraction patterns were recorded in a Debye-Scherres type camera using Ni-filtered Cu radiation and V-filtered Cr radiation. The identity of the unknown diffracting crystallites were determined by visual comparison and by measuring the angular positions of the diffraction lines and comparing them to the values of the cystine standard.

Major Findings: Morphologically two distinct types of birefringent forms were observed in the eyes of patients with cystinosis. Fusiform-shaped bodies have been found predominantly in the cornea and sclera in histologic examinations while the rectangular forms observed were confined to the conjunctiva and uvea.

X-ray diffraction studies indicated that the rectangular forms of crystals seen on histologic examination with regular and polarized light are L-cystine. These crystals were identified in the conjunctiva, peripheral cornea (containing predominantly rectangular forms only), iris, choroid and in the conjunctiva of the two adult patients.

Central corneal tissues and those portions of sclera containing predominantly fusiform bodies gave less conclusive results on x-ray diffraction; cystine diffraction lines were completely absent. These diffraction patterns were consistent with those obtained from control specimens. The uniform negative x-ray diffraction results of corneal tissues containing predominantly fusiform bodies suggests that these birefringent forms are probably not L-cystine crystallites. Additional studies ultilizing micro-amino acid analyses have verified the rectangular crystallites to be cystine but have failed to identify the fusiform bodies with these methods.

The rectangular crystalline forms found in the ocular tissues of childhood and adult forms of cystinosis are identical to the crystals found in the bone marrow of these patients. With the possible exception of a described retinopathy in childhood cystinosis, the ocular manifestations of the two forms of the disease appear similar. The fusiform bodies which were found primarily in the cornea and sclera of the childhood cases and in the corneal biopsy of one adult case remain unidentified. Efforts are continuing in their identification.

Significance to Bio-medical Research and the Program of the Institute: This study represents the first attempt to characterize crystals by x-ray diffraction studies correlated with microscopic observations in eye tissues of patients affected with cystinosis.

Proposed Course of Project: The projected is being continued.

Honors and Awards: None

Publications:

Frazier, P. D. and Wong, V. G.: Cystinosis: histologic and crystallographic examination of crystals in eye tissues. Arch. Ophthal. (in press), 1968.

ANNUAL REPORT
July 1, 1967 through June 30, 1968
Branch of Electroencephalography and Clinical Neurophysiology
National Institute of Neurological Diseases and Blindness

Cosimo Ajmone Marsan, M.D., Chief

Summary of Program Activity

1. Clinical-Diagnostic Service

This service accounts for a considerable portion of the activity of the Branch (about two-thirds of the time of the technical and secretarial staff and over one-third of that of the professional personnel).

A total of 1506 EEG examinations have been performed - between the last report (prepared March 31, 1967) and the present one (prepared April 1st, 1968) - on patients referred from the different Institutes, with the following distribution:

| INSTITUTE | NO. | % |
|---|---|---|
| NINDB | 1004 | 68.6 |
| NCI | 113 | 7.1 |
| NIMH | 103 | 6.3 |
| NHI | 97 | 6.2 |
| NIAMD | 79 | 5.1 |
| NIAID | 77 | 4.7 |
| NIDR, NICHHD and Employee Health Service | 33 | 2.0 |
| | 1,506 | 100.0 |

The number of monthly examinations remains close to that of the last few years (about 125). As in the past the large majority of referrals have originated from our Institute (about 70%) and in particular from the Branch of Neurological Surgery. In addition to the routine (scalp) EEG examinations, 7 records were obtained, in collaboration with the latter Branch, directly from the exposed cortex in the Operating Room in the course of surgical procedures for treatment of seizures.

This diagnostic service is provided either as part of the routine work-up of both in- and out-patients in the Clinical Center or, very often, as part of specific research projects originating both in this Branch and/or Institute and in other Institutes. In the above total of the EEG examinations

are also included a certain number of special examinations carried out by means of chronically implanted electrodes in subjects affected by seizure disorders or, as a preliminary step to therapeutic subcortical lesions, in cases suffering from various forms of involuntary movements (see Report of the Branch of Neurological Surgery). The functioning of this service is now completely satisfactory and the technical staff shortage which in the past two years had somewhat impaired its efficiency is no longer a problem.

2. Research activity

Of the projects that have been completed, one, (begun in the course of the previous fiscal year) has some important bearings on the nature of EEG or, more specifically, on the mechanisms that are involved in the genesis of the gross population potentials. Experiments carried out on the motor cortex of cats and based on a parallel study of the gross response and of the neuronal activity elicited antidromically by stimulation of the isolated pyramidal tract have shown both the negligible contribution of cellular action potentials and the important contribution of the longer duration cellular post-synaptic potentials, - and especially of the inhibitory ones - to gross surface record. This role of post-synaptic potentials in the genesis of the gross potentials is generally recognized; however, the reasons that are usually given, to account for the differential contribution of these two forms of cellular activity to the population potentials, are not always adequate. For instance the matter of temporal and spatial dispersion of unit spikes is not so convincing especially in the case of evoked responses in which a large number of units are known to fire in relatively high synchrony. Thus, on the assumption that other, more fundamental mechanisms might be involved, mathematical theories of electrotonic and volume conduction were used to calculate the extracellular potential fields generated by transients of different duration (i.e., a spike and a PSP) in a model neuron with geometrical and electrical properties of a pyramidal tract cell. The results have indicated that the duration of an intracellular potential is indeed an important parameter (and more so than its amplitude) in the generation of a potential at a remote extracellular point so that a slow, relatively small potential would be expected to give rise to a greater extracellular change than would a brief transient of much greater amplitude. By using the data derived from the study of single units, to construct a population of model cells it has been possible to predict the experimentally observed gross potential distributing throughout the depth of the cortex

during cell discharge and recurrent inhibition.

This project is considered completed. Of the two papers that have been prepared and submitted for publication, one has already appeared in printed form and the other should be published within 1968.

Another research project that had also been initiated in the previous fiscal year has been recently completed, and its results are currently in press. It deals with a study of the anatomical - functional organization of the III nerve nucleus in the cat. Specifically it concerns the distribution of the subnuclei for the Superior Rectus and Inferior Oblique nerves. The data that are available in the literature on this problem are somewhat conflicting. These have been derived mainly from experimental approaches based on either retrograde degeneration following sections of III nerve or on the peripheral effects of gross electrical stimulation within the region of its nucleus. In our study we have used the technique of mapping both gross and unit activity elicited antidromically by stimulation of the whole III nerve and of its branches in the orbit. The results would suggest a preferentially contralateral and caudal localization for the neurons of the superior rectus and a rather scattered, maximally caudal but exclusively ipsilateral localization for the neurons of the Inferior Oblique.

A study related to this same project is currently under way with the main purpose of investigating possible synaptic interactions between the neuronal populations of these two subdivisions of the III nerve.

Two new projects are in the field of experimental epilepsy. One deals with the cellular mechanisms that are at the basis of the characteristic epileptogenic action of strychnine. Previous analogous studies in our Branch had indicated that this drug has the property of affecting the inhibitory post-synaptic phenomena also in the cortical neurons and had suggested the likelihood of a direct effect of the drug on the post-synaptic membrane of these neurons. These results had been obtained by means of gross topical application of strychnine on the cortical surface and because of this type of experimental approach the findings and their interpretations have been recently questioned by other investigators. These claim that, in lower concentrations and with a more discrete form of application, the drug does not exert any detectable effect on the synaptic inhibition of cortical neurons, at least as far as one can judge by data derived with extracellular recordings. Thus, in order to check the validity of such a criticism and/or

that of our original results, the mode of action of strychnine on cortical neurons has been reinvestigated by means of a new experimental approach based on the iontophoretic cellular application of the drug with simultaneous intracellular (and extracellular) monitoring of the synaptic excitatory and inhibitory phenomena of the cortical cell under study. The project is still in its preliminary phases due to the necessity of mastering a number of technical difficulties. Some of the results are however quite promising and would seem to give support to our original interpretation.

The other project also utilizes the micro-electrode (extra- and intracellular) technique and aims at investigating possible differences in the mechanisms of the epileptogenic effects exerted by different pharmacological agents on cortical neurons. Specifically we are interested in determining a) which, if any factor facilitates the occurrence of organized rhythmically recurring ictal episodes (rather than isolated and sporadic discharges of brief duration) and b) what characterizes the neuronal behavior in those situations in which the gross epileptiform potentials are morphologically reminiscent of the human "petit mal" pattern. It is in fact interesting that certain agents (such as, e.g. certain topically applied conjugated estrogens) tend to produce discharges of the 3/sec spike and wave type. This study is currently in progress.

In the field of clinical electroencephalography and always in relation to the various aspects of seizure disorders, we have recently completed a study of a situation that is relatively uncommon in routine EEG; yet,whenever met, it raises serious problems of diagnostic, prognostic and possibly even medico-legal nature. There are certain EEG abnormalities which because of their characteristic features and their tendency to appear in the records of epileptics, are often referred to as "epileptiform". The purpose of our study has been to investigate the incidence and significance of such epileptiform patterns in the records of over 6000 subjects which did not present any clinical evidence for a seizure disorder at the time of their EEG examination.

It has been possible to confirm that such incidence is indeed low (close to 2%). When, however, the non-epileptic patients were subdivided into clinical-diagnostic groups, a significantly higher occurrence of epileptiform discharges could be demonstrated in cases of congenital and perinatal brain damage, brain neoplasms, cranial operations and biochemical disorders. Furthermore, a follow-up study

has shown the subsequent development of clinical seizures in over 14% of the cases. This tendency was found to be particularly prominent in patients with traumatic, vascular and post-operative conditions as well as in cases under anti-neoplastic drug therapy. Of practical interest was the observation that patients with progressive neurological involvement would tend to present their initial seizure episode within one month after the detection of epileptiform discharges in their EEG.

These and other findings have been integrated with those reported sporadically (and often in an indirect form) in the world literature. This project is considered completed. A paper has been submitted for publication and should appear in print in the very near future.

An other similar study in the field of clinical epilepsy has been recently undertaken with the purpose of assessing the factors that might be related to the presence and absence of typical epileptiform EEG manifestations in the records of known epileptic patients. All electroencephalographers are aware of the fact that among subjects that are unquestionably affected by a seizure disorder some show consistently a "positive" (pathognomonic) EEG record, whereas in others serial examinations may occasionally yield records that are "negative" or normal and still in others the EEG remains persistently "negative". Our study attempts to evaluate the role of a large number of factors (such as age, type of epilepsy, seizure frequency, time interval between seizures and EEG, medications etc.) in the determination of the EEG pattern itself. Particular emphasis is placed in the investigation of the group of subjects with totally "negative" records. All possible and pertinent data have been collected in 300 epileptics in which at least 3 and up to over 12 records have been obtained. Such data are now in the process of being analyzed with the cooperation of the Computer and Data Processing Branch of DCRT. The study should be completed within a few months.

The last research project of this Branch is also related to the EEG patterns of epileptics and deals with an analysis of the topographical distribution and spread of epileptiform, inter-ictal "focal" discharges. In scalp electroencephalography, as it applies to patients affected by so-called "focal seizures", one seldom sees a truly "focal" event; this is the case even in the most typical situations in which the epileptiform abnormalities are present and are suggestively predominant over one area of the scalp. A local epileptogenic process (or lesion) is generally assumed to manifest its presence with a local electrical disturbance however a) the size of such a local process varies from

case to case; b) the intracerebral and inter-hemispheral organization varies from area to area and c) volume conductor phenomena are always involved. These and other factors are responsible for the complex and multiform EEG manifestations of focal epilepsy. Our study investigates their distribution and spread utilizing 16 scalp electrodes, magnetic tape recording, digitalization of the electrographic patterns and their analysis with the aid of the LINC computer. Fifty to 100 epileptiform discharges that are interpreted as originating from the main "focus" are averaged and the distribution over the scalp of this averaged potential is evaluated at various intervals of time following its onset. The study, carried out in cooperation with the personnel and the facilities of the Laboratory of Technical Development of the NIMH, is expected to last through part of the next fiscal year.

3. Other activities

The Branch integrates its service activity with the training of clinical electroencephalographers (about two each year). The Branch Chief has continued his part time activity as Chief Editor for America and Australasia of the international Journal "Electroencephalography and Clinical Neurophysiology" and as a member of the editorial committee of the "Archivio di Scienze Biologiche". This activity, though apparently unrewarding and without the display of tangible results as in the case of research projects, is however interesting, quite useful and challenging and contributes greatly to the over-all scientific standing of the Branch and, indirectly, of NINDB and NIH. The Branch Chief is also currently participating in a workshop on Epilepsy (sponsored by NINDB) and is preparing a chapter for the planned volume to be published at the end of the current calendar year. He is in charge of the organization of the VII international congress of Electroencephalography and Clinical Neurophysiology to be held in San Diego, California in 1969. He has also been invited to give the Lennox lecture at the November Meeting of the American Epilepsy Society and serves as Chairman of the Hans Berger award committee of the American Electroencephalographic Society.

4. Programs considered for the near future

Research activity to continue essentially along the same lines. The main interests and general field of investigation of the Branch are not expected to differ significantly from the present ones.

Serial No. NDB(I)-67 EEG/CN 1440
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies of the antidromic cortical response. Cellular bases of the evoked potentials

Previous Serial Number: NDB(I)-67 EEG/CN 1439 and 1440

Principal Investigator: Donald R. Humphrey

Other Investigators: C. Ajmone Marsan

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.2 |
| Professional: | 0.9 |
| Other: | 0.3 |

Project Description:

Objectives, Methods employed, etc.: Our original intent was to investigate the cortical responses to stimulation of the surgically isolated pyramidal tract (PT) of cat, in order to define, unequivocally, the neuronal responses evoked by activity in recurrent axon collaterals. This goal has been accomplished and part of the results have recently appeared in published form. The present project is an outgrowth of this study, and the methods employed are similar to those outlined in the last report. Briefly, our major goal has been to utilize our experimental results and current treatments of electrical potential theory to evaluate recent hypotheses concerning the origins of EEG potentials.

Major findings: Our most significant finding was that the antidromically evoked discharge of PT neurons gave rise to no distinct potential at the surface of the motor cortex, despite the large population of cells involved. On the other hand, recurrent inhibitory potentials (IPSPs) were found to generate prominent surface potentials, even though their amplitudes, when measured intracellularly, were only 1/5 to 1/4 those of cellular

action potentials. In this case there was little or no ambiguity concerning the origins of the various gross potentials; cell discharge and postsynaptic potentials (PSPs) were separable in time, and the contributions of each to the gross record could be clearly assessed. The results thus constituted a particularly convincing demonstration in support of current hypotheses concerning the origin of EEG or cortical surface potentials, viz., that they are generated by slow, graded PSPs in vertically oriented cortical neurons; in previous attempts to test this hypothesis, PSPs were not separable from the discharge of cortical cells. However, a careful analysis of the distribution of single-unit activity over time and cortical depth indicated that the usual explanations advanced in support of the PSP-hypothesis were inadequate (e.g., greater asynchrony of spikes than PSP, cancellation of spike-related currents, etc). Instead, our data suggested that the fundamental mechanism involved a dependence of the potential field around each single neuron on the duration of the intracellular transient. Accordingly, we employed theories of electrotonic and volume conduction to calculate the potential fields generated by transients of different duration, in a model neuron possessing the known geometrical and electrical properties of PT cells. The calculations provided strong, quantitative support for our hypothesis, indicating that the distributions of dendritic membrane current and extracellular potential were increasing functions of the rise time and duration of the intracellular transient. A logical consequence of this hypothesis is that rapid intracellular potentials in a population of cortical neurons will contribute less to gross recordings than long-duration potentials, whether such responses are spontaneous or evoked. By using our single-unit data to construct a population of model cells, we were able to successfully predict the experimentally observed gross potential distributions over cortical depth, during cell discharge and recurrent inhibition.

Significance to Bio-medical Research and the Program of the Institute: As far as we know, this is the first study in which any EEG potential has been quantitatively accounted for, using experimentally derived information concerning the intracellular potentials responsible for generating the observed gross waves. Moreover, our theoretical results account not only for our own data, but provide explanations for previously unresolved or ignored facts concerning the generation of cortical surface potentials and their phase relations to intracellular potentials. Consequently, we feel that we have taken a small but significant step toward placing the study of EEG potentials on more firm theoretical ground than was heretofore possible.

Proposed Course of Project: This project is essentially completed. An additional theoretical paper on the phase relations between EEG and intracellular potentials would be timely and is under consideration.

Honors and Awards: The second publication listed below has been submitted to the American EEG Society for consideration in the Hans Berger Essay Contest.

Publications:

Humphrey, Donald R.: Re-analysis of the antidromic cortical response. I. Potentials evoked by stimulation of the isolated pyramidal tract. Electroenceph. clin. Neurophysiol. 24: 116-129, Feb. 1968.

Humphrey, Donald R.: Re-analysis of the antidromic cortical response. II. On the contribution of cell discharge and PSPs to the evoked potentials. Electroenceph. clin. Neurophysiol. 1968 (In press)

Serial No. NDB(I)-67 EEG/CN 1441
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neurophysiological investigations on the third nerve nucleus in the cat

Previous Serial Number: Same

Principal Investigator: Don C. Bienfang

Other Investigators: C. Ajmone Marsan

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total | 1.2 |
| Professional: | 1.0 |
| Other: | 0.2 |

Project Description:

Objectives: This project was to map the location of the cell bodies of the motoneurons of the superior rectus and inferior oblique muscles by identification of antidromically driven units. In addition, the interaction between this synergistic pair at the neural level is being investigated.

Methods employed: Under barbiturate anesthesia the nerves of the inferior oblique and superior rectus muscle are stimulated. Recording is done with KCl and ferri cyanide micropipettes for the mapping experiments and is being done with K Citrate micropipettes for the study of possible synaptic influences of one nerve upon the other.

Major findings: The mapping of the antidromically driven cells can be considered completed. The principal findings were an ipsilateral and predominantly (but not entirely) posterior position for the inferior oblique motoneurons and a contralateral and mainly posterior position for the superior rectus motoneurons. The study of synaptic interactions of the two nerves has only recently been begun and although some of the

condition-testing experiments would seem to suggest an excitatory influence of the superior rectus upon the inferior oblique motoneurons, results are still preliminary and no definite findings are available.

Significance to Bio-medical Research and the Program of the Institute. The information derived from these two sets of experiments should help understand the process of synergy between the eye muscles and the physiology of the III nerve in general.

Proposed Course of Project: The mapping of the synergistic pair has been completed. The study of the synaptic interaction between these two will continue.

Honors and Awards: None

Publications: Bienfang, D.C.: The location of the cell bodies of the superior rectus and inferior oblique motoneurons in the cat. Exper. Neurol. 1968 (In press)

Serial No. NDB(I)-68 EEG/CN 1541
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cellular mechanisms in experimental epilepsy

Previous Serial Number: None

Principal Investigator: Donald R. Humphrey

Other Investigators: Vazha Okujava (Guest Worker from the Academy of Sciences, USSR) and C. Ajmone Marsan

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives, Methods employed, etc.: Previous studies in our Branch have shown that topically applied strychnine may convert inhibitory postsynaptic potentials (IPSPs) in cortical neurons into depolarizing responses. It was suggested that the epileptogenic action of strychnine may derive from a direct effect on the membranes of cortical neurons, by converting normally inhibitory postsynaptic responses to excitatory ones. These findings have recently been questioned by other investigators, who claim that lower concentrations of strychnine, applied iontophoretically to single cortical cells, have no detectable effect on synaptic inhibition, at least when estimated by extracellular recording data. However, there are actually two problems involved. The first is whether or not strychnine affects inhibitory potentials, and the second is whether or not it affects the inhibitory process; some investigators overlook the fact that these two events are not identical. In view of the importance of this problem for current theories of experimental epilepsy, we have decided to investigate the effects of iontophoretically applied strychnine on synaptic excitation and

inhibition of single cortical neurons. Both intra- and extracellular recording techniques will be employed.

Major findings: This study is in the preliminary stage and very few data have been obtained. Our major efforts so far have been directed at mastering the electrode techniques necessary for this type of experiment, and we feel that we have essentially accomplished this goal.

Significance to Bio-medical Research and the Program of the Institute. An elucidation of the cellular mechanisms involved in epilepsy is one of the major goals of our Branch. We hope that this study, if successful, will shed some light on the problem of whether 'epileptic' neuronal activity derives primarily from altered mechanisms of synaptic transmitter release or from altered properties of the postsynaptic membrane.

Proposed Course of the Project: We shall continue this project on a full-time basis, and hope that the major experiments can be completed within the next six months.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 EEG/CN 1542
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neurophysiological investigations of drug induced cortical epileptic foci

Previous Serial Number: None

Principal Investigators: Russell W. Hardy, Jr.

Other Investigators: C. Ajmone Marsan

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.2 |
| Professional: | 1.0 |
| Other: | 0.2 |

Project Description:

Objectives: The project aims to investigate the characteristics of single unit activity in cortical epileptic foci induced by various pharmacological agents, including penicillin, premarin, neostigmine and acetylcholine.

Methods employed: The experiments are performed on anesthetized cats. Cortical epileptic foci are induced by topical applications of the various pharmacological agents. Single unit behavior is investigated by means of glass microelectrodes. Spontaneous activity of involved cells is recorded and evaluated.

Major findings: The findings, still in the preliminary stage would suggest that (1) frank ictal episodes may be induced by a combination of topically applied penicillin and acetylcholine and (2) premarin may induce seizure foci that differ from those produced by other agents.

Significance to Bio-medical Research and the Program of the Institute. It is hoped that the investigations may yield information that will help to elucidate the cellular mechanisms

responsible for cortical epileptic foci.

Proposed Course of the Project: The project is continued and its completion is expected to take place within the next six months.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 EEG/CN 1438(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Incidence and prognostic significance of "epileptiform" activity in the EEG of non-epileptic patients

Previous Serial Number: Same

Principal Investigators: Lawrence S. Zivin and C. Ajmone Marsan

Other Investigators: None

Cooperating Units: Section on Mathematical Statistics, Office of Biometry, NINDB

Man Years:

| | |
|---|---|
| Total: | 0.8 |
| Professional: | 0.7 |
| Other: | 0.1 |

Project Description:

Objectives, Methods employed, etc.: This study was undertaken to gain information about the occurrence and prognostic significance of epileptiform discharges in the EEGs of patients who had never had a seizure. From the entire EEG file of 9500 patients (21,000 records) and after the examination of the EEGs and medical records of 278 patients 142 were found who met rigid criteria for study purposes. The related literature was extensively reviewed and tabulated.

Major findings: The incidence of epileptiform discharges on EEGs of 2.2% of 6497 non-epileptic patients was broken down into diagnostic groups, which revealed significantly high incidences in patients with congenital and perinatally acquired brain damage, brain neoplasms, cranial operations, mental retardation, biochemical disorders and therapies involving antineoplastic agents and steroids. Twenty (14.1%) of the study patients developed seizures at some time subsequent to the initial epileptiform EEG, most of these patients being under 15 years of age. Patients with deteriorating neuromedical conditions

displayed a marked tendency for seizures to occur, if at all, within one month of the time epileptiform discharges were initially detected, and to have only a single convulsive episode. Patients with chronic and static neurological involvements showed no crucial temporal relationship between detection of epileptiform discharge and seizure occurrence; and seizures, if occurring, tended to do so more than once.

Significance to Bio-medical Research and the Program of the Institute. This is one of numerous NINDB studies related to epilepsy and to the significance of scalp EEG information at a clinically applicable level. The study is of practical interest for diagnostic purposes. Besides permitting the recognition of a latent seizure disorder in the course of syndromes that are not primarily of an epileptic nature, it might also suggest this very nature in certain unsuspected cases.

Proposed Course of the Project: The project is considered completed and a paper has been submitted and accepted for publication.

Honors and Awards: None

Publications: Zivin, L. and Ajmone Marsan, C.: Incidence and prognostic significance of 'epileptiform' activity in the EEG of non-epileptic subjects. Brain, 1968 (In press)

Serial No. NDB(I)-68 EEG/CN 1543
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The presence and absence of epileptiform activity in the EEGs of known epileptic patients

Previous Serial Number: None

Principal Investigators: C. Ajmone Marsan and L. Zivin

Other Investigators: None

Cooperating Units: Division of Computer Research and Technology
Computation and Data Processing Branch

Man Years:

| | |
|---|---|
| Total: | 0.30 |
| Professional: | 0.25 |
| Other: | 0.05 |

Project Description:

Objectives: This study is undertaken to examine the relationships, in known epileptic patients, between the occurrence of EEG information of epileptiform type and numerous clinical factors, such as age, seizure frequency, time since last seizure, medications and type of EEG findings. It is hoped to explore, at a clinical level, some perplexing questions concerning temporal behavior of EEG findings in epileptic patients, and some characteristics of patients, EEG and sampling techniques which may have bearing upon the EEG information ultimately assembled about patients. Particular attention will be paid to patients, all of whose records were negative for epileptiform activity.

Methods: Three hundred patients, randomly selected from the EEG files are being studied. Data tallies and correlations, using single and multiple variant analyses, are being planned with the cooperation of the Computer and Data Processing Branch of DCRT.

Major findings and Proposed Course of the Project: The

results of the various analyses are not available as yet. It is hoped that this analysis will be largely completed within 2-3 months.

Significance to Bio-medical Research and the Program of the Institute. This study should throw some light on the electrographic behavior of epileptics in the inter-ictal periods and should provide information of both diagnostic and pathogenetic nature in the general field of seizure disorders, one of the main interests of our Institute.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 EEG/CN 1553
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Topographical distribution and spread of epileptiform interictal "focal" discharges

Previous Serial Number: None

Principal Investigators: C. Ajmone Marsan and A. J. Gabor

Other Investigators: None

Cooperating Units: Laboratory of Technical Development of NINDB and NIMH

Man Years:

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 0.8 |
| Other: | 0.2 |

Project Description:

Objectives: To investigate the pattern of distribution and spread of "focal" epileptiform discharges as they occur during interictal periods. Under the assumption that these discharges are the result of propagation of electrical discharges of a neuronal population along anatomically definable pathways it is hoped that analysis of these patterns will aid in clarifying and better defining the differences or similarities between epileptiform activity arising in different areas of the brain.

Methods employed: The electrical activity of 16 scalp electrodes is recorded on magnetic tape subsequently digitalized and analyzed with the aid of the LINC computer. Fifty to 100 epileptiform discharges are averaged and the distribution over the scalp of this average potential at various periods of time is evaluated.

Major findings: Inasmuch as only a restricted group of patients can be used for this study and these are not readily

available for evaluation, data are still in the process of being analyzed. Data on 9 patients have been collected over a period of 6 months.

Significance to Bio-medical Research and the Program of the Institute. The project is closely related to one of the major programs of the NINDB being one to further the classification, diagnosis and interrelationships of the various forms of epilepsy.

Proposed Course of the Project: Collect additional data on a larger number of patients.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1967 through June 30, 1968
Laboratory of Neuropathology and Neuroanatomical Sciences
National Institute of Neurological Diseases and Blindness

Igor Klatzo, Acting Chief

## Introduction

The Laboratory of Neuropathology and Neuroanatomical Sciences is a new creation formed by fusion of until then separately existing laboratory units in the respective fields. A belief that these two areas of neurological research logically should cross-fertilize each other constituted one of the main motives for the laboratory's establishment. It is obvious that interpretation of pathological reactions must heavily rely on detailed elucidation of normal structural and functional features. Furthermore, any new data or new techniques developed in neuroanatomical research should be of value for experimental neuropathology and conversely, the findings from the latter discipline magnifying in abnormalities certain structural and functional features may provide clues to their proper interrelationships in the normal brain. Also, a close association between neuropathologist and basic neurobiologist can direct attention of the latter towards general areas and problems which are of great importance for management of human disorders of the nervous system.

At the present time the Laboratory of Neuropathology and Neuroanatomical Sciences consists of 5 sections - 3 dealing predominantly with a broad area of neuroanatomical sciences (Sections on Functional Neuroanatomy, Neurocytology and Experimental Neurology) and 2 concerned primarily with neuropathological problems (Sections on Experimental Neuropathology and Comparative Neuropathology). It is planned that in the future the research programs will be harmoniously balanced between these two respective fields of neurological research. It is also hoped the spirit of collaboration between individual investigators and sections within our Laboratory will considerably increase, since it is reality of modern neurobiological research that many problems in view of their complexity can be successfully elucidated only by application of combined, multidisciplinary techniques and experiences of individual investigators.

In general the research efforts of the Laboratory have been concerned both with achieving further progress in long-term projects and with embarking upon several new territories.

Structural Interrelationships.

The elucidation of fine anatomical interrelationships with particular interest concerning auditory systems has been vigorously pursued during this year. Differences between afferent and efferent auditory endings were studied in the fine ultrastructural detail and a new type of degeneration in axonal endings separated from their cell bodies has been described. Also, fine structure of the olfactory bulb was studied with electron microscope. Special attention was given to certain aberrant auditory and visual pathways which appear to terminate about vessels supplying the nuclear masses of each system. These observations can provide an important structural background to the problems of neurogenic mechanisms regulating regional blood flow of the auditory, visual and other systems. Studies on innervation of blood vessels were conducted also on ultrastructural level.

Studies on autonomic nervous system.

Investigations in this area have been characterized by attempts to correlate the ultrastructural observations with histochemical techniques and particularly with localization of catecholamines by the fluorescence microscopy.

With regard to innervation of the smooth muscle, it was demonstrated that supra- or intravital staining with methylene blue, which could be precipitated by permanganate and observed under the electron microscope, resulted in highly selective uptake of the dye in the cholinergic agranular vesicles. This observation supplies an important criterium for differentiation between cholinergic and adrenergic nerve terminals which can be used in experimental studies on the effects of nerve stimulation and drugs. Permanganate fixation enabled also identification under the E.M. of neurones in the rat brain containing 5-hydroxytryptamine.

Ultrastructural studies on innervation of the sphincter and dilator muscle of the iris revealed that there are two morphologically distinct innervations to each of the respective muscles. New ultrastructural observations on chronological changes in degeneration of the autonomic nervous system were obtained following cervical sympathectomy and ciliary ganglionectomy.

Trophic functions of the peripheral nervous system.

Investigations on the mechanisms by which peripheral nerve influences various metabolic phenomena in the corresponding muscle brought several new data. It could be demonstrated that the influence of nerve on muscle cholinesterase represents a true trophic function of the nerve, independent of nerve impulses, muscle contraction or acetylcholine release. Furthermore, it was shown that the nerve regulates many of the specific chemical constituents of red and white muscle by a trophic mechanism.

Under current investigations is also the role of the nerve in the regulation of qualitative and quantitative aspects of myosin ATPase which is essential in muscle contraction. Trophic dependence of enzymes such as ATPase, acid and alkaline phosphatases on intact innervation was demonstrated unequivocally in experiments in which these enzymes were evaluated in lingual epithelium following interference with the innervation. Thus, nerve effects are being investigated in both motor and sensory systems.

Disease processes such as muscular dystrophy affect intrafusal fiber and red and white extrafusal fibers differentially. In view of the aforementioned observations that the neuron is responsible for many of the specific chemical attributes of these kinds of muscle fiber, an investigation has been begun to elucidate the role of the nerve in the pathogenesis of muscular dystrophy.

Morphological basis for various barrier systems of the brain.

The elucidation of complex barrier systems which regulate and interfere with the passage of substances at various brain interphases has been a major interest in several Sections of this Laboratory. Introduction by Reese and Karnovsky (J. Cell Biol. 34, 207-217, 1967) of horseradish peroxidase as an E.M. tracer has provided a new and elegant technique for observations on passage of proteins of smaller molecular weight in the brain. With regard to brain barrier systems application of peroxidase yielded several important and basic findings. In the mammalian (mice) brain intravenously injected peroxidase does not pass through the endothelium of cerebral capillaries - as it is the case with regard to capillaries of the heart, muscle and "special" areas of the brain (area postrema, etc.). The same holds true for the brain of amphibian salamander where capillaries are endowed with a considerable extracellular space outside of the endothelial tubing. On the other hand, preliminary observations with peroxidase in sharks suggest that these basic permeability relationships may be completely different in Elasmobranchs. In the mammalian choroid plexus the tracer is able to cross the fenestrated endothelium but not the choroidal epithelium. From the CSF the peroxidase is able to pass between cells of the pia-arachnoid layer, however, from the ventricular CSF it does not enter into the median eminence and area postrema. Since passage of a tracer may to a considerable extent depend on various "junction" systems, these have been extensively investigated under high resolution of the E.M. and several new types have been described. Needless to mention that these observations are of basic importance for interpretation of the "barriers" behaviour in abnormal conditions.

Perfection of fixation by perfusion techniques.

Several important factors in successful fixation of the brain have been elucidated in the past year. It was shown that the amounts of fixatives can be greatly reduced when the vascular bed is restricted by clamping of the large blood vessels. With similar considerations a peripheral vasoconstriction was attempted in order to block the peripheral circulation and to funnel solutions exclusively into the central nervous system. A remarkable success was achieved by intracardial injection of epinephrine prior to perfusion. Fixation was restricted to the brain and spinal cord. These observations indicate the possibility of potentiating the action of a pharmacologic agent on the central nervous systems and of minimizing the quantities of expensive radioisotopes for which the brain is the target object. Comparisons between fixations achieved by immersion and by perfusion were carried out in brains subjected to experimental microembolization. These observations might significantly contribute to proper evaluation of various acute neuronal changes encountered in human material.

Cytologic characteristics and reactions of the individual cellular elements of the CNS.

Extensive studies have been carried out on identification of microglial cells under normal and pathological conditions. It is intended to compare reactive mitosis in response to a remote trauma in germ-free and conventional animals in order to elucidate the question of whether the microglial cells are primarily concerned with phagocytic activity and reaction to infections in the CNS.

With regard to reactive mitosis in mesodermal elements, following retrograde neuronal changes evaluation of cortisone revealed that this compound was able to depress the incidence of reactive mitosis, but it did not have any effect on the peripheral chromatolysis in the neurons.

Studies on blood viscosity in macroglobulinemic mice.

Investigations which are of importance to problems of human macroglobulinemia were concerned with the number of aspects related to this condition. In mice it was possible to elucidate the relationships between presence of macroglobulins in the plasma and status of blood viscosity, erythrocyte aggregation, and blood flow in microcirculatory observations. Macroglobulinemia was induced by transplanting a plasma cell tumor into a strain of mice. Animals with greatly elevated blood viscosity displayed remarkable aggregation of erythrocytes in all pial vessels. This was accompanied by visually demonstrable decrease in blood flow.

The contractile response of pial vessels was greatly prolonged in macroglobulinemic mice with elevated blood viscosity. These experiments suggest that the patients with macroglobulinemia may have impaired cerebral blood flow and abnormal microcirculatory responses.

Studies on the brain of Elasmobranch fishes.

The value of comparative studies involving observations on brain of primitive species has been well recognized. Sharks, with 300 million years of recorded history and with some species unchanged for the last 15 million years by evolution, belong to oldest vertebrates. It might be expected that a study of such an evolutionary ancient brain may disclose some basic mechanisms still clearly recognizable before they become covered by complexities of later evolutionary layering. Unique features of cellular morphology, such as perivascular and perineuronal arrangement of astrocytes, argentophilic fibrils investing brain capillaries, etc. reported earlier in the light-microscopic study, prompted extensive E.M. investigations in order to provide basic information on the ultrastructure of the shark brain. In these studies the individual cellular components of the shark brain were ultrastructurally identified and described in detail. The brain capillaries demonstrated several unusual features, the most striking of which was the presence of collagen-containing perivascular space interposed between endothelial tubing and the glial coverings. The mentioned observations on the normal ultrastructure provide the base for investigations on the reaction of the shark brain to various noxious agents. Of special interest appeared to be assessment of radiation damage and such a study has been started at the Lerner Marine Laboratory in Bimini, Bahamas, using a cobalt gamma radiation source. This special interest is related to earlier investigations showing that in the mammalian brain following radiation the most sensitive and earliest changes occur at the glia-blood vessel interphase and involve very early glycogen reaction. Thus, the reaction to radiation in shark brain may be very different in view of basic differences in glia-capillary relationships and glycogen distribution in this animal. Also, the very different metabolic and oxygen consumption rates of the shark are of particular interest since the relationship of circulation and oxygen tension levels to severity of radiation injury are well established. Besides the study of radiation damage, investigations on shark brain include also those related to reactions to traumatic injury, anoxia, etc. Particular attention is given to abnormal glycogen reactions about which a considerable data in mammals has been obtained from our previous studies on radiation in rats, perinatal asphyxia, ischemia, etc.

Studies on brain edema.

During the past year studies on brain edema were confined to completion of extensive biochemical assay of various enzymes and proteins in experimental cold injury and to a collaborative project with the Surgical Neurology Branch on correlation between the size of extracellular spaces assessed by impedance measurements in various types of experimental brain edema. Impedance measurements generally were in agreement with behaviour of extracellular compartment suggested by other approaches such as E.M. and biochemical assays. The models of brain edema were: 1) due to cold injury, 2) due to microembolization, and 3) due to triethyl poisoning.

Studies on brain glycogen.

Brain glycogen which can be demonstrated by histochemical, biochemical and E.M. techniques is closely related to glucose metabolism - the basic fuel of the brain. The striking, abnormal accumulations of glycogen following various types of brain injury are of great interest since their elucidation may lead to explanation of the role played by various nervous tissue cellular components in normal and abnormal glucose transport and metabolism. Of considerable value is a micromethod for quantitative assay of glycogen in thin paraffin embedded sections which has been developed recently in one of our sections. In stab wound injuries of the brain histochemically the glycogen appeared to be abnormally increased in astrocytes confined to close vicinity of lesion, but quantitative microchemical measurements showed increased glycogen many millimeters from the lesion as well. The quantitative method, moreover, reveals that the increased glycogen persists for a longer time than it could be suspected from histochemical observations. This microchemical method offers a great promise for our planned investigations on behaviour of glycogen in perinatal asphyxia in the new-born monkeys, in anoxia and ischemia in cats, and in various injuries of the shark brain. In a separate study a normal distribution of glycogen in neurons of the brain and spinal cord was investigated in various mammalian species.

Serial No. NDB(I)-68 LNNS/CN 1544

1. Neuropathology & Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Localization of Prolactin in Cultured Cells of the Rostral Pars Distalis of the Pituitary of Fundulus Heteroclitus (Linnaeus).

Previous Serial Number: None.

Principal Investigator: E. W. Emmart, Ph.D.

Other Investigators: M. J. Mossakowski, M.D., and Igor Klatzo, M.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMD, and Surgical Neurology Branch, NINDB

Man Years:

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|---|---|
| Total: | 0.6 |
| Professional: | 0.2 |
| Other: | 0.4 |

Project Description:

Objectives: This project was undertaken primarily with the purpose to assess technical possibility of culturing in vitro piscine brain tissues and of applying fluorescent antibody technique for localization of hormonal substances in such cultures. A successful accomplishment of such undertaking would provide a base for using these complex and difficult approaches in our experimental studies on elasmobranchs fishes. In particular, it may enable us to localize and immunochemically define various protein components of the shark brain in perspective of philogenetic relationships.

Methods Employed: F. heteroclitus which had been maintained in aerated synthetic sea water for 3-6 months, were anaesthesized with 0.07% tricane methane sulfonate in sea water. The pituitary gland was removed under aseptic conditions and set in cultures on collagen clots with addition of nutrient medium. Antibody to ovine prolactine was prepared in rabbits, tested by the precipitin method and conjugated to fluorescein isothiocyanate. The cultures were incubated with fluorescent antibody and as a control procedure normal globulin, also conjugated to fluorescein isothiocyanate, was applied. Cultures were photographed in the phase and fluorescence optics. They were also stained with specific for pituitary methods and examined under the light microscope.

Major Findings: Within a week after explantation the pituitary cells began to divide and migrate outward in sheet-like formation. Frequently isolated cells became detached from the parent explant and formed new colonies. The specific granules (stained with Orange G or erythrosin) appeared to fill the cytoplasmic area. Cultures to which the fluorescent antibody solution has been applied showed the light green fluorescent antigen-antibody complex in large granules which were both irregular in size and shape. This did not occur in cultures treated with normal fluorescent globulin. Specific immunochemical staining of prolactin showed a good correlation with morphological appearance of the cells. Preparations of the whole culture stained with fluorescent antibody also showed areas of prolactin cells which contained no prolactin and also variations in the intensity of fluorescent staining both in the center of the explant as well as in the peripheral outgrowth. Studied under phase-contrast optics the living in culture prolactin cells of fundulus have shown the emmission of small particles from the undulating and rippling membrane of the living cell. These swelled rapidly and disappeared.

Significance to Bio-medical Research and the Program of the Institute: This investigation carried out entirely within the facilities in our Laboratory proved that it was possible to grow successfully cold-blooded piscine pituitary tissue which has retained a hormone producing ability in vitro. Application of fluorescent antibody allowed selective and specific localization of this hormone within cytoplasmic structure. This work should be considered as the step providing technical experience for future studies on identification of various protein substances in brain tissues grown in vitro and especially with application to marine species with their wealth of neurosecretory mechanisms.

Proposed Course of the Project: This project is completed.

Honors and Awards: None.

Publications:

Emmart, E. W. and Mossakowski, M. J.: The Localization of Prolactin in Cultured Cells of the Rostral Pars Distalis of the Pituitary of Fundulus heteroclitus. General and Comparative Endocrinology, 9, 391-400, 1967.

Serial No. NDB(I)-68 LNNS/CN 1545
1. Neuropathology & Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neurofibrillary Tangles Induced by Vincristine and Vinblastine Sulfate in Central and Peripheral Neurons in Vitro.

Previous Serial Number: None.

Principal Investigator: Fredrick J. Seil, M.D.

Other Investigators: Peter W. Lampert, M.D., and Igor Klatzo, M.D.

Cooperating Units: Armed Forces Institute of Pathology, Washington, D. C.

Man Years:

| | |
|---|---|
| Total: | 1.2 |
| Professional: | 1.0 |
| Other: | 0.2 |

Project Description:

Objectives: An accidental discovery that intracerebral injection of minute amounts of aluminium phosphate may result in a widespread neurofibrillary degeneration, similar to that observed in various degenerative diseases of the CNS in man, has opened a wide area for experimental investigations on pathogenic mechanisms involved in this abnormal process. (Klatzo, I., Wisniewski, H. and Streicher, E.: Experimental Production of Neurofibrillary Degeneration. J. Neuropath. & Exp. Neurol. 24, 187-199, 1965). Besides aluminium phosphate several other compounds have been found capable of inducing similar degeneration. Production of neurofibrillary tangles in nerve cells grown in tissue culture has advantage of allowing a systematic study of various stages in the development of the tangles. Neurofibrillary tangles and neuronal degeneration were induced by vincristine and vinblastine sulfate in cultures of fetal rabbit spinal cord and dorsal root ganglia.

Methods Employed: Fetal rabbit spinal cord and dorsal root ganglia were grown in vitro on collagen coated coverslips together with a drop of nutrient medium. Cultures were given their initial exposure to vincristine sulfate and vinblastine sulfate at 17,20,21,23 and 26 days in vitro. The drugs were given in the nutrient medium in concentrations ranging from 0.5 nanograms to 20 nanograms per drop of medium. Exposure time varied from 31 hours to 14 days. Control cultures fed with normal nutrient medium were observed for time periods equivalent to those of the treated cultures.

Cultures were observed in the living state under bright-field optics,

under phase contrast and with polarized light. Fixed, whole-mount preparations were stained with thionine and with silver techniques specific for demonstration of neurofibrillary tangles. Cultures of dorsal root ganglia destined for electron microscopic study were fixed for 10-15 minutes in 1% osmium, dehydrated, embedded in Epon and sectioned with a Porter-Blum microtome.

Major Findings: Neurofibrillary tangle development began as early as two days after exposure to as little as 0.5 nanograms of vincristine sulfate per culture, but generally approximately a week was required for full development of large tangle formations. Nine days of exposure to vinblastine sulfate followed by six days in normal nutrient medium resulted in return of both spinal cord and dorsal root ganglia neurons to nearly normal appearance, while sister cultures fixed after nine days in vinblastine treatment exhibited neurofibrillary tangles.

Tangle formation was initiated by a slight thickening of the normally delicate neurofibrils followed by a condensation of argentophilic material into regular or irregular perinuclear halos. The progressive thickening of the argentophilic zone led eventually to the full development of the neurofibrillary tangle.

Under E.M. the affected neurons contained bundles of closely packed neurofilaments which measured about 100 Å in width. Higher resolution of transversely cut filaments revealed a clear central core suggesting a tubular ultrastructure.

Significance to Bio-medical Research and Program of the Institute: This study adds two more compounds to those reported as able to produce experimental neurofibrillary tangles. The substances reported previously include aluminium phosphate, aluminium hydroxide and colchicine. The possibility of studying the formation of neurofibrillary tangles *in vitro* as demonstrated in the present investigation and demonstration of reversibility of these changes under experimental conditions contributes further to elucidation of mechanisms involved.

Proposed Course of the Project: This project is completed.

Honors and Awards: None.

Publication:

Seil, F. J. and Lampert, P. W.: Neurofibrillary Tangles Induced by Vincristine and Vinblastine Sulfate in Central and Peripheral Neurons in Vitro. Experimental Neurology. In Press.

Serial No. NDB(I)-68 LNNS/CN 1546

1. Neuropathology & Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Correlation between the size of Extracellular Spaces Assessed by Impedance Measurements and the Various Types of Experimental Edema.

Previous Serial Number: None.

Principal Investigator: Choh-luh Li, M.D.

Other Investigators: Barbro Johansson, M.D., and Igor Klatzo, M.D.

Cooperating Units: Surgical Neurology Branch, NINDB

Man Years:

| | |
|---|---|
| Total: | 1.2 |
| Professional: | 0.8 |
| Other: | 0.4 |

Project Description:

Objectives: Evaluation of data derived from various experimental models of brain edema has led to classification of this process into two main types, namely - vasogenic and cytotoxic. (Klatzo, I.: Presidential Address. J. Neuropath. & Exp. Neurol. 26, 1-14, 1967). It has been postulated that vasogenic type, related to a primary injury of blood vessels and their leakage, is associated with a dilatation of extracellular spaces, whereas the cytotoxic type, depending on cellular swelling of various morphological components of brain parenchyma, is connected either with reduction or lack of change in extracellular compartment. Although the E.M. observations have widely confirmed the enlargement of extracellular spaces in vasogenic edema, technical aspects of electron microscopy make it difficult to demonstrate changes of extracellular compartment in the cytotoxic type. The present study was undertaken to make such correlations using new and improved methods for measurement of electrical impedance from which it is possible to estimate the size of extracellular compartments.

Methods Employed: The experimental models of brain edema were as follows: 1) edema produced by application of cold metal plate to the exposed cortex of the cat, 2) edema due to embolization produced by intracarotid injection of paraffin material in cats and 3) Triethyl tin edema produced in cats.

Cold lesion edema served as the classic model of vasogenic type of edema.

Its various parameters have been extensively studied in this Laboratory. Edema due to embolization is localized primarily in the cerebral cortex and in the other regions of grey matter. Triethyl tin edema has been previously extensively studied in various laboratories. It affects primarily the white matter.

Following inducement of edema in cats, specific resistivity of the cortex and the white matter was determined, action potentials and resting potentials were recorded from single nerve cells in the cerebral cortex and spontaneous electrical activity and transcollosal evoked responses were registered.

For observations on the behaviour of the blood-brain barrier, animals were injected with Evans Blue prior to sacrifice. The brains were fixed by perfusion. The microscopic distribution of the Evans Blue tracer was assessed under the fluorescence microscope. The paraffin embedded material was subjected to various histological stains.

Major Findings: In the cold injury edema the electrophysiological measurements in the affected white matter showed a marked decrease of impedance amounting to a drop by approximately 100% in comparison with the measurements in the normal white matter. Impedance measurements in triethyl tin poisoned cats showed slightly increased values in the grey matter and virtually no change of impedance in the white matter. In cases of edema produced by paraffin emboli there was about 50% increase of impedance in the cortex. These impedance changes after embolization were extremely rapid in onset and preceded any histological changes characteristic for edematous process. The blood-brain barrier changes and histological features of edemas were similar to those previously described. The impedance measurements allowed a fine assessment of the dynamics of the edema process.

Significance to Bio-medical Research and Program of the Institute: This study represents another contribution in a series of investigations concerning cerebral edema, which is of major interest in the Section on Comparative Neuropathology. Electrophysiological measurements of the extracellular compartment in various types of edema were generally in agreement with evaluations by other approaches, specifically those by electron microscopy. Lack of impedance changes in the white matter in cats poisoned with triethyl tin agrees with data by Streicher (J. Neuropath. & Exp. Neurol. 21, 437, 1962), who demonstrated that thiocyanate space does not change in triethyl tin edematous cats. The electrophysiological measurements when properly applied and analyzed may have potential value in clinical evaluation of patients suspected of having brain edema. Sensitivity of these measurements may provide more intimate assessment of dynamics of developing or subsiding brain edema.

Proposed Course of the Project: This project requires further supplement of data and particularly to fill the gap pertaining to early changes assessed by both electrophysiological measurements and morphological observations.

Honors and Awards: None.

Publications: None.

Serial No. NDB(I)-68 LNNS/CN 1547
1. Neuropathology & Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effects of Ionizing Radiation on the Elasmobranch Brain.

Previous Serial Number: None.

Principal Investigator: Igor Klatzo, M.D.

Other Investigators: Arland L. Carsten, Ph.D.,
Milton Brightman, Ph.D., and Yngve Olsson, M.D.

Cooperating Units: Medical Department, Brookhaven National Laboratory,
Upton, Long Island, N. Y.

Man Years:

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 0.5 |
| Other: | 1.5 |

Project Description:

Objectives: Recent studies have shown the shark brain to exhibit a profound and unusual resistance to changes in vascular permeability and blood-brain barrier following severe local injury (Klatzo, I. and Steinwall, O.: Observations on Cerebrospinal Fluid Pathways and Behaviour of the Blood-Brain Barrier in Sharks. Acta Neuropath. 5, 161-175, 1965). The present investigation is to determine if such a resistance to radiation effects might be also present, and if so, what are the factors causing such a resistance (comparative data is available on mammalian species).

The unique features of cellular morphology such as perivascular arrangement of astrocytes, the ultrastructural characteristics of glia-capillary junctions, distribution of glycogen, etc. create a special interest for studying effects of radiation in Elasmobranchs brain, since it has been shown before that in the mammalian brain the most sensitive changes occur at the glia-blood vessel interphase and involve an abnormal glycogen reaction (Klatzo, et al.: Effects of Alpha Particle Radiation on the Rat Brain Including Vascular Permeability and Glycogen Studies. J. Neuropath. & Exp. Neurol. 20,459-483, 1961). Also, the very different metabolic and oxygen consumption rates of the shark are of particular interest since the relationship of circulation and oxygen tension levels to severity of radiation injury are well established.

Methods Employed: Approximately 50 nurse sharks have been exposed to a well collimated gamma ray beam directed at the cerebellum and underlying portions of the hypothalamus, tegmentum and pons. The radiation source consisting of the cobalt bomb was shipped from the Brookhaven National Laboratory to the Lerner Marine Laboratory, Bimini, Bahamas, and was installed there. The dose range was 1000 - 30,000 r. 25 sharks were irradiated (1000 - 5000 r.) in December 1967 and sacrificed 4 months later. The remaining sharks were subjected to study of acute effects of radiation with dose ranging up to 30,000 r. and were sacrificed within first few days following irradiation. Corresponding in dose radiation was given to cats and those were sacrificed at the comparable time intervals. For the observations on the behaviour of the blood-brain barrier sharks and cats were injected intravenously with the Evans Blue-albumin tracer, usually several hours before the sacrifice. The brains were perfused through the heart by buffered solutions of paraformaldehyde or fixatives designed for the electron microscopy and processed for histological, histochemical and ultrastructural studies.

Major Findings: This project is still in a very early stage. The tissues from the animals irradiated in Bimini have been shipped to Bethesda and are being currently processed. The gross observations on the behaviour of blood-brain barrier revealed that cats which received 30,000 r. and were sacrificed a few days later showed distinct extravasation of Evens Blue tracer in the irradiated areas, whereas this was not observed in sharks which were subjected to similar dose of radiation and were sacrificed at the corresponding time intervals.

Significance to Bio-medical Research and the Program of the Institute: The Section on Comparative Neuropathology, Laboratory of Neuropathology and Neuroanatomical Sciences, is vitally interested in research on elucidation of some basic pathogenic mechanisms in the nervous system. It is felt that studies on primitive vertebrate and invertebrate brain by confrontation of differences found under various experimental conditions offer a unique opportunity to expand our understanding of intricate neurobiological structure-function relationships. This particular project should contribute to elucidation of various patho-mechanisms operative in the radiation damage of the human brain.

Proposed Course of the Project: This project is at the present time in the preliminary stage and will be pursued towards its completion by the investigators involved.

Honors and Awards: None.

Publications: None.

Serial No. NDB(I)-68 LNNS/CN 1548
1. Neuropathology & Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Ultrastructural Features of the Shark Brain.

Previous Serial Number: None.

Principal Investigator: Don M. Long, M.D., Ph.D.

Other Investigators: Thomas S. Bodenheimer, M.D., J. Francis Hartmann, Ph.D., and Igor Klatzo, M.D.

Cooperating Units: Department of Neurology, University of Illinois, Chicago, Ill.; Surgical Neurology Branch, NINDB.

Man Years:

| | |
|---|---|
| Total: | 1.5 |
| Professional: | 0.5 |
| Other: | 1.0 |

Project Description:

Objectives: The recent light microscopic studies on the cellular morphology of the shark brain (I. Klatzo: Cellular Morphology of the Lemon Shark Brain, In: Shark, Skates and Rays. Edit. by P. W. Gilbert, R. F. Mathewson and D. P. Rall., Johns Hopkins Press, Baltimore, 1967) brought out several unique features which undoubtedly must be related to special functional characteristics and to previously reported differences in reactivity of shark brain to injury (Klatzo, I. and Steinwall, D.: Observations on the Cerebrospinal Fluid Pathways and Behaviour of the Blood-Brain Barrier in Sharks. Acta Neuropath. 5, 161-175, 1965). Some of the most interesting morphological features relate to the reverse than in mammals perivascular arrangement of astrocytes, to clustering of glial cells around blood vessels and neurons and to presence of argentophilic fibers around the capillaries of the brain. So far a comprehensive study on the ultrastructure of Elasmobranchs brain has been entirely lacking and this investigation was undertaken to provide a basic information which would be of obvious importance for our future studies concerning behaviour of shark brain under various abnormal experimental conditions.

Methods Employed: This study is based on material prepared for light microscopy and electron microscopy from 16 sharks (hammerhead, tiger, and nurse species). The sharks were provided by the Marine Station of the Department of Marine Biology, University of Puerto Rico, La Parguera, Puerto Rico;

fixation and tissue processing were carried out at this facility. The fixation was carried out by intravascular perfusion with combination of glutaraldehyde and formaldehyde in shark buffer. For ultrastructural localization of sodium the tissue was fixed by immersion in 1% unbuffered osmium followed by an osmium-antimony mixture. Some tissue prior to dehydration was stained with uranyl acetate. Sections were stained with lead citrate and examined in RCA EMU-3F and EMU-3E electron microscopes.

Major Findings: The individual cellular elements of the shark brain were ultrastructurally identified and described in detail . The CNS capillaries of sharks demonstrated several unusual features, the most striking of which was the presence of collagen-containing perivascular space interposed between endothelial tubing and the glial coverings. In many instances, particularly in young sharks, there was a striking evagination of the perivascular space into the adjacent glial processes. In immature sharks a capillary is occasionally totally invested by single glial cell process rather than two or more as in the mammal. In certain parts of the brain the pericapillary glia contained tightly packed filaments, reminiscent of mammalian glial filaments but far more extensive in array. Sodium antimonate precipitate was found in astrocytes in large quantities and in smaller amounts in neurons and their processes.

Significance to Bio-medical Research and the Program of the Institute: This study provides a base-line for our future investigations of various pathogenic mechanisms in Elasmobranchs. Description of normal ultrastructural characteristics will immensely facilitate the understanding of difference from mammalian shark brain reactions to various pathogenic agents and types of injury.

Proposed Course of the Project: The major part of this project is completed. This will be later supplemented by further studies, particularly involving use of E.M. demonstrable tracers such as peroxidase.

Honors and Awards: None.

Publications:

Long, D. M., Bodenheimer, T. S., Hartmann, J. F. and Klatzo, I. : Ultrastructural Features of the Shark Brain. American Journal of Anatomy, In Press.

Serial No. NDB(I)-62 LNNS/ENP 942
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Histochemical study of nerve cells

Previous Serial Number: LN/EN 942

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.0 |
| Professional: | 0.0 |
| Other: | 0.0 |

Project Description:

Objectives: To discern the distribution of glycogen-containing neurons.

Methods Employed: Rabbit brains are fixed in situ and prepared according to methods developed in this Section.

Major Findings: There are through the spinal cord and brain stem two types of motor nerve cells which are distinguished by varied histochemical composition.

Significance: The identification of biochemical differences between neurons may give a basis for understanding functional and pathologic differences in reaction.

Proposed Course of Project: Microscopic examination of the histologic material which has been prepared by various histochemical enzyme digestion methods.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-62 LNNS/ENP 944
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Hematologic control of primates

Previous Serial Number: LN/EN 944

Principal Investigator: Mrs. Margaret G. Johnson

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.3 |
| Professional: | 0.0 |
| Other: | 0.3 |

Project Description:

Objectives: To clarify the reason for poor health in primates.

Methods Employed: Different primate species are regularly examined by various hematologic techniques; weight and dietary regimen are controlled.

Major Findings: Many of the primates suffer from severe infections and aberrations of blood composition.

Significance: A careful hematologic examination is required prior to subjecting primates to experiments.

Proposed Course of Project: Regular check of primates and control of infectious condition.

A long term study of the hematologic status in healthy animals.

A long term study of the effect of varied dietary regimen and adjuvants on the hematologic status.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-63 LNNS/ENP 1065
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Perfection of the perfusion technique for fixation in situ.

Previous Serial Number: LN/EN 1065

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.3 |
| Professional: | 0.3 |
| Other: | 1.0 |

Project Description:

Objectives: To specify quantities of solutions needed to obtain adequate fixation of the brain and spinal cord.

Methods Employed: Deeply anesthetized animals, in some of which the aorta and subclavian arteries were clamped, were perfused with different quantities of a fixative under varying pressures.

Major Findings: The amounts of fixatives can be greatly reduced when the vascular bed is restricted by clamping of the large blood vessels.

Significance: Since many of the most efficient fixatives are expensive, it is of importance that the quantities needed can be limited to a minimum.

Proposed Course of Project: Completed. Manuscript ready for publication.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-63 LNNS/ENP 1066
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Acute degenerative changes in the central nervous system

Previous Serial Number: LN/EN 1066

Principal Investigator: Jan Cammermeyer

Other Investigators: Alden W. Dudley, Jr. (Research Associate, 1963-1965)

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.0 |
| Professional: | 0.0 |
| Other: | 0.0 |

Project Description:

Objectives: To compare the changes in material fixed by immersion and by perfusion.

Methods Employed: Experimental microembolization was carried out in various animal species which at different post-operative stages were sacrificed by the perfusion procedure developed in this Section.

Major Findings: A complex of neuronal changes develops in the foci of degeneration.

Significance: To identify the specific acute neuronal changes and to correlate them with those encountered in clinical neuropathology material.

Proposed Course of Project: Microscopic examination is awaiting the histologic preparation of already completed experiments.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 LNNS/ENP 1237
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cytologic characteristics of microglial cells

Previous Serial Number: LN/EN 1237

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.4 |
| Professional: | 0.1 |
| Other: | 0.3 |

Project Description:

Objectives: To establish the identity of microglial cells under normal and pathologic conditions, and the mode of development of these cells.

Methods Employed: Microglial cells were studied in material fixed by perfusion with Bouin's solution (using the submerged heart method) and in sections stained by a silver carbonate method modified here.

Major Findings: There is a considerable regional and species variation in appearance of microglial cells.

They are widely distributed throughout the central nervous system at all ages.

These cells during their development undergo a series of changes, which have not previously been recognized.

Significance: There is considerable controversy concerning the identity and the role of microglial cells. The recognition of microglial cells throughout the brain and spinal cord of all mammals studied suggests that they form an integral part of the central nervous system, and that they are indispensable for the functional capacity of its various elements.

Proposed Course of Project: Completed. All observations are being compiled for a general review on the microglial cells.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 LNNS/ENP 1449
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The effect of cortisone on the retrograde neuronal changes.

Previous Serial Number: LN/EN 1449

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.0 |
| Professional: | 0.0 |
| Other: | 0.0 |

Project Description:

Objectives: To determine what effect cortisone treatment may have on the acute reactive phase and the subsequent recovery phase of motor neurons subsequent to transection of their axons.

Methods Employed: Cranial motor nerves were cut in different animal species. Serial sections of the brain stem were stained by a combined PAS-gallocyanin method.

Major Findings: Preliminary studies have revealed that the acute peripheral chromatolysis is not influenced by cortisone treatment. The incidence of reactive mitosis is depressed at the five day stage when, however the neuronal changes appear to be uninfluenced.

Significance: In the acute stage of retrograde changes, cortisone has been found to have a selective action on the mesodermal elements. Since such treatment affects protein metabolism it is to be expected that in later stages, when protein synthesis of neurons is increased, the recovery phase of neurons would be affected, too. The results of this study would form a basis to evaluate the usefulness of cortisone treatment during conditions of nerve regeneration.

Proposed Course of Project: Microscopic analysis of the histologic material.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 LNNS/ENP 1450
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Post-operative incidence of mitosis after transection of the facial nerve.

Previous Serial Number: LN/EN 1450

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.2 |
| Other: | 0.5 |

Project Description:

Objectives: To determine age and species differences in incidence of reactive mitosis.

Methods Employed: Counting of mitotic cells in the facial nucleus of animals fixed by perfusion with the submerged heart method. Both conventional and germ-free animals are utilized. Serial sections of the brain stem are stained by the combined method of PAS and gallocyanin.

Major Findings: After the operation, the sequence of changes in incidence of reactive mitosis varies with age and species. The microglial cells are equally well developed in conventional and germ-free animals.

Significance: When the intensity of mesodermal reaction, as evidenced by incidence of mitosis, to an experimental procedure or noxious agent is being estimated, both the age and species must be taken into account.

Since from earlier studies, it has been shown that the microglial cell is the principal cell evolving from mitosis, it follows that the demand for and the ability to develop microglial cells vary with age and species. The question of whether the microglial cells are primarily concerned with phagocytic activity and reaction to infections in the central nervous system may be solved by a comparison of reactive mitosis in response to a remote trauma in germ-free and conventional animals.

Proposed Course of Project: Partially completed. Some of this material will be included in a review on microglial cells (Serial No. NDB(I)-65 LNNS/ENP 1237).

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 LNNS/ENP 1451
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effects of anticoagulants on the viscosity of normal blood.

Previous Serial Number: LN/EN 1451

Principal Investigator: William I. Rosenblum

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: To determine the effects of anticoagulants on blood viscosity.

Methods Employed: Blood and plasma viscosity, and hematocrit were measured in mouse, rabbit or monkey blood anticoagulated with citrate, oxalate, EDTA, ACD solution, or heparin. Red blood cells were suspended in sodium chloride solutions of varying tonicity; suspension viscosity and hematocrit were measured.

Major Findings: Citrate and oxalate shrank erythrocytes (decreased the hematocrit) and elevated the viscosity. Heparin, ACD or EDTA were anticoagulants which did not shrink erythrocytes, and when these agents were employed, blood viscosity was not elevated. The importance of cell shrinkage was further illustrated by the fact that the viscosity of red blood cell suspensions could be raised by adding the erythrocytes to increasingly hypertonic solutions of sodium chloride.

Significance: In order to study the effects of disease on blood viscosity, anticoagulants like heparin or EDTA should be used. Citrate or oxalate may cause elevations in viscosity which would lead to erroneous interpretations of the effects of disease. In addition to this practical consequence of the work, the experiments appear to supply a model system for further study of the effects of altered erythrocytes on blood viscosity.

Current interest in this area is indicated by the appearance of at least two other confirmatory reports since completion of this study. These papers also indicate that erythrocyte shrinkage raises blood viscosity. The hypothesis currently favored to explain this phenomenon is one which relates increased viscosity to an increased "stiffness" of the erythrocytes, produced by their shrinkage.

Proposed Course of Project: The project is completed.

Honors and Awards: None

Publications:

Rosenblum, W. I.: In vitro measurements of the effects of anticoagulants on the flow properties of blood: The relationship of these effects to red cell shrinkage. Blood 31: 234-241, Feb. 1968.

Serial No. NDB(I)-67 LNNS/ENP 1452
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Blood viscosity in macroglobulinemic mice

Previous Serial Number: LN/EN 1452

Principal Investigator: William I. Rosenblum

Other Investigators: Richard M. Asofsky

Cooperating Units: NIAID, LGAR

Man Years

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.1 |
| Other: | 0.1 |

Project Description:

Objectives: To answer the following questions: a) Is blood viscosity altered in macroglobulinemic mice? b) What are the causes of the alteration? c) Do these mice provide a useful model for the study of factors affecting blood flow in human macroglobulinemia?

Methods Employed: Macroglobulinemia was produced in Balb/c mice by implanting plasma cell tumor MOPC 104E subcutaneously in the abdomen. This tumor secretes macroglobulin into the blood stream. Blood, serum or plasma was obtained from these mice and from normal controls and the viscosity of these fluids was determined using a capillary viscosimeter. The viscosity of blood was related to the hematocrit and to the concentration of macroglobulin in the serum or plasma. The concentration of macroglobulin was determined by immunodiffusion. Macroglobulinemic serum or plasma also served as a source of purified macroglobulin, prepared by ultracentrifugation. This macroglobulin was added to suspensions of normal red blood cells. The viscosity of these suspensions was measured and was related to the concentration of macroglobulin in the suspending medium and to the hematocrit of the suspension. In addition, the effect of macroglobulin on erythrocyte morphology was examined by microscopic observation of blood streaming between a glass slide and cover slip.

Major Findings: Blood viscosity was elevated in macroglobulinemic mice. The elevation was caused by the presence of macroglobulin which acted in at least two ways. First, plasma viscosity was elevated and this raised the viscosity of whole blood. Second, macroglobulin greatly increased the tendency of erythrocytes to adhere to one another, and the resultant aggregates greatly elevated blood viscosity. The effects of macroglobulin were accentuated at high hematocrits and partially ameliorated by low hematocrits.

Significance: Data from these mice resembles that gathered by other workers in their studies of human macroglobulinemia; therefore these animals appear to provide a valuable model of the human disease. Our experimental observations emphasize the importance of erythrocyte aggregation in the pathogenesis of increased blood viscosity. Moreover, these aggregates may be expected to impede blood flow in the microcirculation and to contribute, along with increased viscosity per se, to the production of the transient and varied symptoms appearing in macroglobulinemic patients. Since aggregation is not assayed by measurement of plasma or serum viscosity, measurement of blood viscosity would appear to be a preferable technique for evaluating clinical status.

Proposed Course of Project: Our intention to inject purified macroglobulin into normal mice was thwarted by our inability to purify sufficient amounts of this material. Therefore this project was terminated. However, observations made in the course of a related project (NDB(I)-67 LNNS/ENP 1453) led to additional investigations of factors controlling blood viscosity in macroglobulinemia. Description of the latter appears in a separate project report (NDB(I)-68 LNNS/ENP 1581).

Honors and Awards: None

Publications:

Rosenblum, W. I., and Asofsky, R. M.: Factors affecting blood viscosity in macroglobulinemic mice. J. Lab. Clin. Med. 71: 201-211, Feb. 1968.

Serial No. NDB(I)-67 LNNS/ENP 1453
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Relationship of blood viscosity to the behavior of the cerebral microcirculation in macroglobulinemic mice.

Previous Serial Number: LN/EN 1453

Principal Investigator: William I. Rosenblum

Other Investigators: Richard M. Asofsky

Cooperating Units: NIAID, LGAR

Man Years

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.1 |
| Other: | 0.1 |

Project Description:

Objectives: To determine the effects of elevated blood viscosity on the behavior of the intracranial microcirculation.

Methods Employed: In vivo microscopy of pial vessels was carried out according to methods previously developed by the investigator. Vasospasm was induced by local applications of $BaCl_2$ and the magnitude and duration of response were determined. Responses in macroglobulinemic mice were compared with those in normal mice. Macroglobulinemia was produced by transplanting a plasma cell tumor MOPC 104E into Balb/c mice. Blood viscosity was measured with a capillary viscosimeter. Immunodiffusion was used to determine the concentration of macroglobulin in the plasma.

Major Findings: Animals with greatly elevated blood viscosity displayed remarkable aggregation of erythrocytes in all pial vessels. This was accompanied by a visually demonstrable decrease in blood flow. Animals with lesser elevations of blood viscosity failed to display in vivo aggregation of erythrocytes. Aggrega-

gation and slowed flow was especially pronounced in a group of macroglobulinemic mice which had been dehydrated for 24 hours. These animals had extremely high viscosities even when they displayed only moderately high macroglobulin levels.

The contractile response of pial vessels was greatly prolonged in macroglobulinemic mice with elevated blood viscosity. It was not prolonged in animals bearing other types of plasma cell tumors, and having normal blood viscosities.

Significance: Transient neurologic symptoms occur in patients with macroglobulinemia during periods of elevated blood viscosity. These experiments suggest that the patients may have impaired cerebral blood flow and abnormal microcirculatory responses.

Proposed Course of Project: The data indicates that cerebral microcirculation is abnormal in macroglobulinemia, a condition characterized by increased blood viscosity. The project is completed and an additional project has been undertaken to determine whether similar observations can be made in other vascular beds, and whether other hyperviscous states are accompanied by identical microcirculatory disturbances.

Honors and Awards: None

Publications:

Rosenblum, W. I., and Asofsky, R. M.: Malfunction of cerebral microcirculation in macroglobulinemic mice. Arch. Neurol. 18: 151-159, Feb. 1968.

Rosenblum, W. I.: Blood viscosity and cerebral microcirculation. In Siekert, R. G., and Whisnant, J. P. (Eds.): Sixth Princeton Conference on Cerebrovascular Diseases, Jan. 1968. New York, Grune & Stratton. In press.

Serial No. NDB(I)-67 LNNS/ENP 1454
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neuropathologic study of macroglobulinemic mice.

Previous Serial Number: LN/EN 1454

Principal Investigator: William I. Rosenblum

Other Investigators: None

Cooperating Units: NIAID, LGAR

Man Years

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: To determine whether histologic abnormalities of the nervous system are induced by macroglobulinemia.

Methods Employed: The brains and spinal cords of mice suffering from macroglobulin-producing tumors have been fixed in situ and prepared histologically according to methods developed in this Laboratory for an analysis of different central nervous system elements.

Major Findings: No abnormalities were found. Animals were also given to Henry Schutta, M.D., University of Pennsylvania, Department of Neurology, for electron microscopic investigation. His data also failed to reveal morphologic evidence of damage to the central nervous system.

Significance: The negative results are not surprising in view of the fact that macroglobulinemic mice die within 2 months, usually without definite evidence of neurologic dysfunction. However, there is also a paucity of significant morphologic findings in macroglobulinemic humans even after a long course

including transient neurologic symptoms. Therefore, the negative histologic data may only serve to emphasize the importance of functional microcirculatory abnormalities, like those described in a related project (NDB(I)-67 LNNS/ENP 1453).

Proposed Course of Project: Completed.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 LNNS/ENP 1455
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Investigations of the effect of a lead-rich diet on the central nervous system in suckling mice

Previous Serial Number: LN/EN 1455

Principal Investigator: William I. Rosenblum

Other Investigators: Margaret G. Johnson

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.1 |
| Other: | 0.6 |

Project Description:

Objectives: To determine whether histologically demonstrable lesions are present in the nervous system of lead poisoned neonatal animals.

Methods Employed: Pregnant Balb/c mice were placed on a lead containing diet immediately after delivering their litters. Their progeny thereby suckled milk containing lead. At various intervals, brains and spinal cords were fixed in situ and prepared according to methods developed in this Laboratory for the demonstration of different elements in the brain.

Major Findings: Lead-fed neonates displayed a marked retardation in growth and development and a very high mortality. Study of the nervous system revealed astrocytosis and microglial proliferation or enhanced silver impregnation of scattered foci of microglial cells and foci of astrocytes in the absence of neuronal alterations, parenchymal softening or hemorrhage. An increase was noted in the number of intervascular strands of connective tissue.

Significance: Changes may represent an effect of lead on the brain but may also represent the effects of multiple abnormalities partially reflected by impaired growth and development.

Proposed Course of Project: Project completed after technical difficulties precluded electron probe analysis of tissue for lead.

Honors and Awards: None

Publications:

Rosenblum, W. I., and Johnson, M. G.: Neuropathologic changes produced in suckling mice by adding lead to the maternal diet. Arch. Path. In press.

Serial No. NDB(I)-68 LNNS/ENP 1577
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The interpyramidal glio-vascular region

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.4 |
| Professional: | 0.1 |
| Other: | 0.3 |

Project Description:

Objectives: The identification of structures along the surface of central nervous sytem.

Methods Employed: Paraffin embedded material fixed by perfusion with Bouin's solution was stained by different histologic techniques and a silver carbonate method modified here for differential staining of blood vessels, myelin sheaths, neurons and microglial cells with oligodendrocytes.

Major Findings: Where a large cerebral artery sends off branches to the medulla oblongata, the midline sulcus contains numerous astrocytic elements, collagen fibers and microglial cells. This region is separated from adjacent brain stem regions by collagen fibers.

A similar region is identified in sloth, rodent, rabbit, carnivores and primates.

Significance: Further studies are required to determine the significance of this region. Tentatively only a mechanical function for attachment of blood vessels has been ascribed to this region.

Proposed Course of Project: Completed.

Honors and Awards: None

Publications:

Cammermeyer, J.: The interpyramidal glio-vascular region. Z. Anat. Entwicklungsgesch. 126: 332-341, 1968.

Serial No. NDB(I)-68 LNNS/ENP 1578
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The use of epinephrine for selective fixation of the brain and spinal cord.

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.3 |
| Professional: | 0.2 |
| Other: | 0.1 |

Project Description:

Objectives: To apply peripheral vasoconstriction to the perfusion procedure for fixation of the brain and spinal cord in order to block the peripheral circulation and to funnel solutions exclusively into the central nervous system.

Methods Employed: Perfusion of different animals first with saline and then with Bouin's solution. The amounts of fluids are determined by flow from graduated perfusion bottles and the duration of flow is timed with a stop watch. Epinephrine is injected intracardially prior to perfusion.

Major Findings: Fixation is restricted to the brain and spinal cord; however the retina, the sympathetic ganglia, the adrenals and the thyroid gland are also fixed.

Fixation of the central nervous system can be obtained with very small amounts of fixative without cumbersome surgical intervention by clamping of the aorta and the subclavian arteries.

Significance: By this new principle of perfusion fixation, the amounts of expensive agents employed can be greatly reduced, which is especially significant when larger animals are being used. If a similar principle may be applied to other types of experiments, this may prove helpful as a means to potentiate the action of a pharmacologic agent on the central nervous system and to minimize the quantities of expensive agents such as radio-isotopes.

Proposed Course of Project: Completed. Manuscripts on the effect in different species and on the procedure to be followed are being readied for publication.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 LNNS/ENP 1579
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Rotating slide holder for microscope

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.1 |
| Other: | 0.1 |

Project Description:

Objectives: To construct a device permitting proper orientation of a slide on a microscopic stage whereby coaxial movements of the stage can be performed with exact reference to certain structures in the microscopic section.

Methods Employed: Device was constructed in metal by the Instrument Shop according to a sketch of the instrument.

Major Findings: Microscopic sections of different size can be scrutinized after being properly oriented.

Significance: Slide can be rotated 360° to allow counting of cells, photographic reconstruction of tissue and photometric studies of different parts of the cerebral cortex.

Proposed Course of Project: Completed.

Honors and Awards: None

Publications:

Cammermeyer, J.: A rotating slide holder for the mechanical stage of a microscope. Stain Technol. 00:000-000, May 1968.

Serial No. NDB(I)-68 LNNS/ENP 1580
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effects of dehydration on blood viscosity in macroglobulinemia.

Previous Serial Number: NONE

Principal Investigator: William I. Rosenblum

Other Investigators: Richard M. Asofsky

Cooperating Units: NIAID, LGAR

Man Years

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: To determine the reasons for the elevation of blood viscosity which follows dehydration of macroglobulinemic mice.

Methods Employed: Macroglobulinemic was produced by transplanting plasma cell tumor MOPC 104E into Balb/c mice. One group of macroglobulinemic animals was deprived of water for 24 hours. Deprived and non-deprived animals were bled and blood viscosity determined with a capillary viscosimeter. Microhematocrits were obtained. The Biuret procedure was due to determine total plasma protein. Immunodiffusion was used to measure macroglobulin levels in the plasma. Non-macroglobulin levels were determined by subtracting macroglobulin concentration from that of total protein.

Major Findings: Dehydration increased blood viscosity by raising the hematocrit and the concentration of macroglobulin. In addition the total protein increased, but this elevation was due largely to the augmented macroglobulin levels. In fact, non-macroglobulin concentration diminished. The shift in relative concentrations of various plasma proteins may also

contribute to the elevated blood viscosity since such shifts may affect the degree of red cell aggregation in macroglobulinemic blood.

Significance: Osmotic factors appear to be important in controlling the relative concentrations of plasma proteins in macroglobulinemia. The large macroglobulin molecule apparently connot leave the vascular compartment readily, while other protein fractions move out of the vessels and lessen the detrimental increase in plasma osmolarity which accompanies accumulation of macroglobulin. This may be true not only during dehydration, but also in non-deprived macroglobulinemics, since in these animals there was also an inverse relationship between macroglobulin or total protein levels on the one hand, and non-macroglobulin levels on the other. Similar phenomena have been reported in humans with macroglobulinemia.

Proposed Course of Project: Completed.

Honors and Awards: None

Publications:

Rosenblum, W. I., and Asofsky, R. M.: Effects of dehydration on blood viscosity and on distribution of plasma proteins in experimental macroglobulinaemia. Nature 216: 1327-1328, Dec. 1967.

Serial No. NDB(I)-68 LNNS/ENP 1581
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The relationship of erythrocyte aggregation to microcirculatory malfunction in hyperviscous states.

Previous Serial Number: NONE

Principal Investigator: William I. Rosenblum

Other Investigators: None

Cooperating Units: NIAID, LGAR

Man Years

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.1 |
| Other: | 0.1 |

Project Description:

Objectives: To answer the following questions: a) Is microcirculatory malfunction manifest in more than one vascular bed in macroglobulinemia? b) Can a "physiologic" vasoconstrictor agent produce an abnormally prolonged contractile response in the vessels of macroglobulinemic animals? c) Do polycythemic mice demonstrate the same microcirculatory dysfunction as macroglobulinemic animals? If not, to what factors can the difference be ascribed?

Methods Employed: Macroglobulinemic mice were prepared as previously indicated (NDB(I)-67 LNNS/ENP 1452). Polycythemic mice were prepared by multiple intraperitoneal transfusions of red blood cells. Hematocrit and blood viscosity measurements were performed. The subcutaneous vessels of living macroglobulinemics were microscopically observed. Norepinephrine was used to constrict subcutaneous vessels. Duration of constriction was compared with that in normal animals. Data was compared with earlier work showing prolonged vasoconstriction in pial

vessels of macroglobulinemic animals. Locally applied $BaCl_2$ was used as a pial vasoconstrictor. Pial microcirculation in macroglobulinemic mice was also compared with that of polycythemic animals.

Major Findings: Prolonged vasoconstriction was demonstrated in subcutaneous vessels of macroglobulinemic mice, as well as in the pial microcirculation. Erythrocyte aggregation was observed in subcutaneous vessels and was enhanced during vasoconstriction. Aggregates appeared to impede reentry of blood into constricted subcutaneous vessels. Pial vessels in polycythemic mice showed no functional abnormalities. Findings were negative in spite of viscosity values in excess of those found in macroglobulinemia.

Significance: Abnormal vasoconstriction is observed in several vascular beds of macroglobulinemic mice and is elicited by physiologic as well as non-physiologic stimuli. These findings are not related to elevated blood viscosity per se, since even higher viscosity values are found in polycythemia, but without demonstrable microcirculatory malfunction. Abnormal flow and contractile responses appear related to the presence of erythrocyte aggregation, which is a feature of macroglobulinemia but not of polycythemia. The aggregates are one cause of elevated blood viscosity in the former disease. The data emphasizes the importance of distinguishing between hyperviscous states on the basis of their causation.

Proposed Course of Project: Completed. Manuscript written and submitted for publication.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 LNNS/ENP 1582
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effect of low molecular weight dextran on blood viscosity in macroglobulinemia.

Previous Serial Number: NONE

Principal Investigator: William I. Rosenblum

Other Investigators: None

Cooperating Units: NIAID, LGAR

Man Years

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: To determine whether low molecular weight dextran (D-40) is efficacious in reducing blood viscosity in macroglobulinemia.

Methods Employed: Blood was obtained from macroglobulinemic mice (NDB(I)-67 LNNS/ENP 1452). D-40 was added to one aliquot, dextrose and saline was added to another. Viscosity of all aliquots was determined with a capillary viscosimeter. Hematocrit measurements assured equal dilution of experimental and control samples.

Major Findings: A large and significant elevation of viscosity was produced by the addition of D-40.

Significance: Many workers have advocated therapeutic use of D-40 in a variety of disease states. The rationale has been that D-40 reduces blood viscosity because it is a specific means of inhibiting erythrocyte aggregation. Other workers dispute this view, and suggest that any benefits following the use of D-40 are merely the result of hemodilution. The present data is derived from blood known to have an abnormal propensity for aggregation.

The results suggest that this aggregation was not inhibited, and since viscosity was greatly increased, therapy with D-40 is contraindicated in macroglobulinemia. This data is compatible with studies from other institutions, indicating that if hematocrit is held constant, D-40 will elevate the viscosity of normal blood.

Proposed Course of Project: Completed.

Honors and Awards: None

Publications:

Rosenblum, W. I.: Effects of dextran-40 on blood viscosity in experimental macroglobulinaemia. Nature. In press.

Serial No. NDB(I)-68 LNNS/ENP 1583
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neuropathologic changes in sulfite oxidase deficiency

Previous Serial Number: NONE

Principal Investigator: William I. Rosenblum

Other Investigators: None

Cooperating Units: NCI, PA; NIAMD, A & R

Man Years

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: To determine the nature of neuropathologic changes in a case of a newly defined metabolic disease.

Methods Employed: The brain and spinal cord of a 3-year-old child were examined grossly and microscopically with a variety of techniques. During life the child showed severe neurologic dysfunction and was found to have a deficiency of the enzyme, sulfite oxidase.

Major Findings: Severe lesions of variable age were found in white and gray matter. The deep white matter displayed diffuse loss of myelin and axons, with relative sparing of the zone of arcuate fibers. Sudanophilic myelin breakdown products were present. Scattered cysts were found in the deep white matter, amid areas of rarifaction and astrocytosis. The overlying cortex was often well demarcated from these zones by the better preserved region of arcuate fibers. The cortex itself displayed many scattered areas of degeneration with varying degrees of neuronal loss and astrocytosis. Similar findings were seen in the thalamus, basal ganglia, and cerebellar nuclei, accompanied by cyst formation in these areas.

Significance: The lesions are non-specific and cannot be related at this time to sulfite oxidase deficiency. The pattern of white matter destruction may be related to an antecedent cerebral edema of uncertain origin. The clinical and pathologic findings resemble those found in some cases of so-called cystic encephalomalacia of infancy. Many such cases have been classified as cerebral palsy. Future analysis of such cases for sulfite oxidase deficiency, coupled with post mortem examination, will determine which, if any, of the present morphologic abnormalities are related to the enzymatic deficiency.

Proposed Course of Project: Completed. Manuscript submitted for publication.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 LNNS/ENP 1584
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Investigations of barium's action as a vasoconstrictor

Previous Serial Number: NONE

Principal Investigator: William I. Rosenblum

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: To determine the mechanism by which $BaCl_2$ produces vasoconstriction.

Methods Employed: A spiral strip of rat aorta was suspended in physiologic solution and attached to an isotonic recording transducer. Shortening was recorded on an Offner dynograph, and calibrated in terms of milligrams tension required to produce this degree of shortening. A variety of drugs were added to the bath in an effort to interfere with barium's contractile action.

Major Findings: The vasomotor action of barium is altered by a variety of agents.

Significance: Previous work has demonstrated that locally applied $BaCl_2$ will produce profound constriction of cerebral blood vessels, less than 100 micra in diameter. Because such vessels are not readily constricted by more "physiologic" materials this response to $BaCl_2$ was of some interest since it indicated that contractile "machinery" was indeed present in these small arteries and arterioles. The present study was designed to shed light on what the contractile mechanism might be, by utilizing

a larger vessel, more accessible to isolation and in vitro manipulation. However, the rat aorta selected for these studies, unexpectedly displayed properties which distinguish it from other vascular tissue. Therefore, further investigations of the pharmacology of this preparation will be necessary in order to understand barium's mode of action.

Proposed Course of Project: This investigation has to be terminated here because of the departure of the investigator from NIH, but will be continued by him at his new post outside the PHS.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-63 LNNS/EN 1054
1. Neuropathology and Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: "Trophic" functions of the peripheral nervous system

Previous Serial Number: LNS/EN 1054

Principal Investigator: Lloyd Guth

Other Investigators: R. Wayne Albers and Frederick Samaha

Cooperating Units: Laboratory of Neurochemistry, NINDB

Man Years

| | |
|---|---|
| Total: | 2.2 |
| Professional: | 0.7 |
| Others: | 1.5 |

Project Description:

Objectives: To investigate the various mechanisms by which the peripheral nerve influences the metabolism of its end-organ. Previous studies have shown that the nerve specifically regulates the cholinesterase activity of the muscle fiber. Experiments have therefore been performed to determine whether the nerve acts by way of impulse transmission, acetylcholine release or by some unknown (trophic) mechanism. Other possible trophic influences are also being investigated as part of this project, viz., the influence of the nerve on enzymes of carbohydrate metabolism and on ATPase activity of myosin.

Methods: (1) Sole-plate and background cholinesterase activity were measured in muscles following these operations: a. denervation near or far from the muscle; b. spinal cord transection; c. tenotomy; d. denervation plus physiotherapy; e. immobilization of the ankle joint by internal fixation. (2) Cross-union of nerves to crureus (red) and rectus femoris (white) in the cat and of nerves to soleus (red) and medial gastrocnemius (white) in the rat were performed. Several months later quantitative determinations of a variety of enzymes of

carbohydrate metabolism were made. (3) Biochemical techniques for distinguishing quantitatively and qualitatively between the myosin ATPase activity of red and white muscle have been developed for future study on the role of the nerve in the regulation of the ATPase activity of muscle.

Major Findings: (1) a. Whether the nerve was cut near or far from the muscle the rate of loss of ChE was identical. Since miniature end-plate potentials (random, quantally-released acetylcholine) diminish more rapidly when the nerve is severed near the muscle, this result suggests that ACh release is not important in the neural regulation of ChE. b. Spinal cord transection produced less of a decrease in ChE than did spinal cord transection in combination with denervation. Since spinal cord transection is virtually without effect on miniature end-plate potentials, this is further evidence that the quantally-released ACh is unrelated to the neural regulation of ChE. It also indicates that influences from higher centers may affect the trophic function of the lower motor neuron. c. Neither tenotomy nor immobilization, both of which procedures produce an electromyographically quiescent muscle, resulted in any loss in ChE. Consequently, muscle contractions are not believed to be of primary importance in the maintenance of ChE. (2) Reinnervation of a red muscle by a nerve normally destined for a white one resulted in an increase in those chemical constituents characteristic of white muscle. This was true for enzymes of carbohydrate metabolism, isozymes of lactic dehydrogenase and electrophoretically-separated soluble proteins. (3) Characteristic qualitative and quantitative differences between the myosin ATPase of red and white cat muscles have been demonstrated.

Significance: (1) Inasmuch as the influence of nerve on muscle cholinesterase is unrelated to nerve impulses, muscle contraction or acetylcholine release, it would appear that this influence constitutes a true trophic function of nerve. As a result of this data, future studies on the mechanism of the trophic effect may be attempted. (2) It is apparent that the nerve regulates the chemical constituents of red and white muscle in a highly specific fashion. However, because the pattern of impulses transmitted by nerves to red and white muscle is different, we cannot be sure whether or not this effect is mediated by nerve impulses. Future experiments will be aimed at studying the role of nerve impulses, muscle work, and trophic influences in the regulation of the specific biochemical attributes of red and white muscle. (3) Because myosin is the major single protein constituent of muscle, and because myosin ATPase is uniquely involved in the contractile process, further studies on the role of the nerve in the

regulation of qualitative and quantitative aspects of myosin ATPase may prove of great promise in the study of trophic nerve function. Studies of myosin ATPase following cross-reinnervation of red and white muscles are under way.

Proposed Course of Project: As indicated in the preceding paragraph, these studies will be continued during the coming year.

Honors and Awards: None

Publications:

Guth, L., Brown, W. C. and Watson, P. K.: Studies on the role of nerve impulses and acetylcholine release in the regulation of the cholinesterase activity of muscle. Exper. Neurol. 18: 443-452, Aug. 1967.

Guth, L., Watson, P. K. and Brown, W. C.: Effects of cross-reinnervation on some chemical properties of red and white muscles of rat and cat. Exper. Neurol. 20: 52-69, Jan. 1968.

Serial No. NDB(I)-66 LNNS/EN 1303
1. Neuropathology and Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Histo- and biochemical characteristics of muscle sensori - motor components

Previous Serial Number: LNS/EN 1303

Principal Investigator: Herbert Yellin

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.7 |
| Professional: | 1.0 |
| Others: | 0.7 |

Project Description:

Objectives:

1. To study morphological and histochemical characteristics of sensory receptors in normal and atypical vertebrate muscle.

2. To study, by histochemical methods, the neural regulation of muscle enzymes.

3. To study by histo- and biochemical methods the role of innervation in the manifestation of mammalian skeletal muscle dystrophy.

Methods Employed:

1. Normal, denervated, tenotomized and dystrophic muscle are being studied by standard histological and histochemical techniques, particularly those demonstrating the disposition and relative levels of glycolytic and oxidative enzymes.

2. Randomly reinnervated and doubly innervated muscles are being studied histochemically for their glycolytic and oxidative enzyme characteristics.

3. Muscles, differentially affected by the dystrophic process are being studied for their myofibrillar, sarcoplasmic and stromal content (a) under usual circumstances and (b) following reinnervation by alien neurons.

Major Findings:

1. Several enzyme profiles are observed among the intrafusal fibers of rat muscle, and only some conform to the enzyme profiles of extrafusal muscle. Chronic muscle denervation results in variable atrophy and loss of enzyme activity of intrafusal fibers. The decline in intrafusal fiber activity may parallel that of the rapidly atrophying extrafusal fibers, though intrafusal fibers with more persistent enzyme activity are observed. A normal complement of spindles and intrafusal fibers is found in the gastrocnemius (white) and soleus (red) muscles of dystrophic mice. Intrafusal fibers of dystrophic (and chronically tenotomized) muscles appear to retain their structure and enzyme activity for extended periods, despite severe atrophy and enzyme loss of surrounding extrafusal fibers.

2. Extrafusal muscle fibers enzymatically characteristic of either red or white rat muscle undergo conversion of enzyme profiles as a result of alien reinnervation.

Significance:

1. The presence in multi-innervated intrafusal fibers of enzyme profiles different from those of the singularly innervated extrafusal muscle fibers may be attributable to differences in both number and type of innervating neurons.

2. The energy-deriving enzyme systems and physiological capabilities of extrafusal muscle fibers are under neural regulation. Speed of contraction of muscle can be correlated roughly with muscle fiber enzyme types. It appears quite clear that neither the chemical nature of red (slow) and white (fast) muscle, nor the physiological properties of slow and fast muscle are immutable, but that they are maintained throughout life by virtue of a still undisclosed neural mechanism.

3. In a variety of primary myopathies there is apparent differential involvement of extrafusal muscle fibers and general sparing of the intrafusal fibers of muscle spindles. If the efferent innervation regulates the contraction-kinetics and energy metabolism of muscle fibers then the following questions arise; (a) Do qualitatively differing innervations protect some muscle fibers from the intrinsic disorders of striated muscle,

and (b) are a number of primary muscle diseases dependent upon specific neural influences for their manifestation? If the skeletomotor innervation was a predisposing factor in the relative expression of some primary myopathies, then the nervous system might serve as the vehicle for pallative or remedial measures.

Proposed Course of Project: This project will continue as a major portion of the coming year's work.

Honors and Awards: None

Publications:

Yellin, H.: Neural regulation of enzymes in muscle fibers of red and white muscle. Exptl. Neurol. 19: 92-103, Sept. 1967.

Eldred, E., Yellin, H., Gadbois, L., and Sweeney, S.: Bibliography on muscle receptors; Their morphology, pathology and physiology. Exptl. Neurol., Suppl. 3, pp. 1-154, 1967.

Serial No. NDB(I)-68 LNNS/EN 1585
1. Neuropathology and Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cerebral glycogen metabolism following brain injury

Previous Serial Number: None

Principal Investigator: Lloyd Guth

Other Investigator: Igor Klatzo

Cooperating Units:

Man Years

| | |
|---|---|
| Total: | 0.8 |
| Professional: | 0.3 |
| Others: | 0.5 |

Project Description:

Objectives: Following brain injury glycogen is known to accumulate in astrocytes. The purpose of the present investigation was to develop quantitative microtechniques for glycogen assay that would permit correlation of histochemical observations with quantitative measurements. A quantitative and histochemical description of the sequence of changes in glycogen is necessary before beginning studies on the metabolic turnover (synthesis and degradation) of glycogen following brain injury.

Methods Employed: A micromethod for glycogen based on the enzymatic conversion of glycogen to 6-phosphogluconic acid and the reduction of TPN to TPNH was employed. Fluorimetric assay of TPNH formation provides an accurate estimation of glycogen content in tissue samples.

Major Findings: Glycogen is fully preserved in paraffin sections of rat brain, provided that perfusion fixation is performed without development of anoxia and provided the brain is dehydrated solely in the higher grades of alcohol. By perfusing with buffered paraformaldehyde, and by initiating dehydration with 90% alcohol - 10% formalin mixtures we have

preserved normal glycogen content and have obtained histochemical preparations that are far superior to those obtained following fixation in the usually recommended alcohol-picrate mixtures. Following stab wound injury of the brain the glycogen increases mainly in the region of the injury, but quantitative measurements revealed increased glycogen many millimeters from the lesion as well. The quantitative method, moreover, reveals that the increased glycogen persists for a longer time than was suspected from histochemical observations.

Significance: The successful application of quantitative methods to fixed tissue specimens will permit a variety of studies that have heretofore been impossible. Because glycogen decreases so rapidly following the onset of anoxia, the only previous method for preserving glycogen was by rapid freezing -- a procedure feasible solely in small animals such as the mouse. Studies on the reaction of brain tissue to injury are now possible in larger animals (e.g., monkey and chimpanzee) in which the experimental conditions more nearly correspond to those in man.

Proposed Course of Project: To be continued during the current year.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 LNNS/EN 1586
1. Neuropathology and Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Trophic nerve function as related to degeneration, regeneration, and cytodifferentiation

Previous Serial Number: None

Principal Investigator: Andrew A. Zalewski

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.3 |
| Professional: | 1.0 |
| Others: | 0.3 |

Project Description:

Objectives: To investigate the neural influence on enzymes in taste organs.

Methods Employed: (1) Bilateral and unilateral nerve sections were performed on the circumvallate papilla of rats. Changes in enzymes in the taste buds (adenosine triphosphatase and acid phosphatase) and in the surrounding epithelium (alkaline phosphatase) were studied histochemically and quantitatively at various intervals after denervation.

Major Findings: Alkaline phosphatase which is located in the superficial stratified epithelium only in taste bud areas disappeared almost completely after bilateral denervation. No change occurred after unilateral denervation.

Significance: This experiment shows the trophic dependence of an enzyme (alkaline phosphatase) on an intact innervation. This finding supports current concepts that the neurotrophic influence may be mediated via the regulation of cellular enzymes. It may be that through neural enzyme regulation cytodifferentiation and regeneration occur.

Proposed Course of Project: Future studies will deal with the specificity of neural regulation of enzymes in taste organs.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-61 LNNS/NC 808
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The innervation of smooth muscle

Previous Serial Number: LNS/NC 808

Principal Investigator: Keith C. Richardson

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.5 |
| Professional: | 0.5 |
| Others: | 0.0 |

Project Description:

Objectives: To study the ultrastructure of autonomic nerve terminals on smooth muscle in collaboration with chemical and pharmacological investigations.

Methods Employed: Standard electron microscopical techniques of fixation and epoxy resin embedding, using neonatal and adult rat tissues.

Major Findings: Recent evidence obtained by fluorescence microscopy for the localization of catecholamines, and histochemical techniques for acetylcholinesterase, which can be applied to the same preparation, indicates that in many situations of the autonomic nervous system there is a dual contribution of cholinergic and adrenergic axons to the innervation of smooth muscle. As shown previously, this dual innervation is demonstrated electronmicroscopically by two distinct populations of vesicles, granular and agranular, in the respective axons. A further contribution to the identification of the axons has now been made with methylene blue vital staining.

When methylene blue is applied supra-vitally or intra-vitally

to living tissues like the iris, it has been shown by Ehinger, Sporrong and Stenevi (Life Sciences, 6, 1967, 1973) that the nerves stained form a network which differs in location and in pattern from the fluorescent adrenergic network revealed by the Falck and Hillarp technique in the same preparation. This light microscopical finding has been confirmed electron-microscopically by fixing vitally stained tissue in permanganate. The tissue was then found to contain axons which were altered in fine structure by swelling and derangement of the axoplasm, together with electron dense deposits beneath the axolemma caused by interaction of the dyestuff with the permanganate. These altered axons invariably contained agranular vesicles which labelled them as cholinergic. The adrenergic axons containing granular vesicles were entirely unaffected by the vital staining.

The methylene blue effect appears to be confined to the terminal regions of the cholinergic axons which evidently show a highly specific uptake mechanism for the dyestuff which is absent in the adrenergic axons.

Significance: As a basis for experimental work on the effects of nerve stimulation and drugs, it is necessary to have reliable indicators of cholinergic and adrenergic axons at the ultra-structural level. It is believed that this new approach with methylene blue may be superior to the previous combination of acetylcholinesterase histochemical staining, because the latter technique does not favor the simultaneous demonstration of granular vesicles in the adrenergic axons.

Proposed Course of Project: To resume experimental studies on the effects of nerve stimulation and drugs on the fine structure of adrenergic, peripheral nerve terminals.

Honors and Awards:

Publications: Richardson, K. C.: Cholinergic and adrenergic axons in methylene blue-stained rat iris: an electronmicroscopical study. Life Sciences (In press)

Serial No. NDB(I)-66 LNNS/NC 1304
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Pinocytosis in the brains of dead rats

Previous Serial Number: LNS/NC 1304

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

This project is being held in abeyance as the work will have to be reinvestigated with new staining techniques and a new marker (Peroxidase).

Serial No. NDB(I)-67 LNNS/NC 1443
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The intracerebral movement of proteins injected into the blood and cerebrospinal fluid of rodents

Previous Serial Number: LNS/NC 1443

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.4 |
| Professional: | 0.4 |
| Others: | 0.0 |

Project Description:

Objectives: To examine morphologically the blood-CSF barriers of the choroid plexus to proteins.

Methods Employed: Horseradish peroxidase (6.0 mg) was injected into the femoral vein of mice whose brains were then fixed at different intervals (15 to 20 min.) and examined electron microscopically.

Major Findings: Peroxidase was able to cross the fenestrated endothelium but not the epithelium of the choroid plexus. The protein was incorporated though not transported in appreciable amounts by numerous pinocytotic vesicles. The extracellular movement of peroxidase into the ventricles was stopped by tight junctions between the epithelial cells.

Significance: The structural barriers to the passage of protein from blood into the ventricle reside in the epithelium of the choroid plexus.

Proposed Course of Project: To extend the observations on movement of peroxidase both from the blood and from the CSF and to determine the role of vesicular transport across the

fenestrated endothelium.

Honors and Awards: None

Publications:

Brightman, M. W.: Movement within the brain of ferritin injected into the cerebrospinal fluid compartments. In Klatzo, I. and Seitelberger, F. (Eds.): Brain Edema. New York, Springer-Verlag, 1967, pp. 271-284.

Bodenheimer, T. S., and Brightman, M. W.: A blood-brain-barrier to peroxidase in capillaries surrounded by perivascular spaces. Am. J. Anat. (In press)

Serial No. NDB(I)-67 LNNS/NC 1445
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The electronmicroscopic identification of adrenergic neurons and nerve terminals in the C.N.S.

Previous Serial Number: LNS/NC 1445

Principal Investigator: Keith C. Richardson

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.4 |
| Professional: | 0.4 |
| Others: | 0.0 |

Project Description:

Objectives: To identify the ultrastructural characteristics of the perikarya and axon terminals of neurones in the brain of the rat containing norepinephrine or 5-hydroxytryptamine.

Methods Employed: Permanganate fixation, epoxy resin embedding or freeze substitution.

Major Findings: The identification of adrenergic terminals in rat brain has been anticipated by Hokfelt (Zeit. Zellforsch., 79, 1967, 110) who has found that neurones in the locus coeruleus contain small and large granular vesicles in the perikaryon as well as in synaptic terminals. This work confirms the superiority of permanganate fixation for catacholamine-containing vesicles which are not uniformly preserved by glutaraldehyde or osmium tetroxide fixation. However, the identification of neurones containing 5-hydroxytryptamine by electronmicroscopy is still under investigation. Intestinal enterochromaffin cells and blood platelets have been found to contain large granular vesicles indicating that a reaction between 5-HT and permanganate is a possible means of

identification of structures known to contain 5-HT. So far similar granular vesicles have not been identified in the nucleus suprachiasmatieus or in regions of the brain stem where fluorescence microscopy demonstrates the presence of 5-HT. The problem is complicated by the low concentration of 5-HT in these neurons as judged by fluorescence microscopy, and the inevitable loss of the transmitter with fixation. Experiments are in progress using the freeze substitution method of van Harreveld and Crowell which it is hoped will overcome these difficulties.

Significance: The identification of perikaryia and synaptic terminals in brain associated with the presence of norepinephrine, dopamine and 5-hydroxytrptamine at the ultrastructural level is of great importance in future experimental work involving identification of nuclei and tracts as well as the effects of drugs on these structures. Progress has been slow because of the technical difficulties involved.

Proposed Course of Project: Further investigation with various new methods of freeze substitution and dehydration without fixation.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 LNNS/NC 1447
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: An ultrastructural study of iris innervation after autonomic ganglionectomy in albino rats

Previous Serial Number: LNS/NC 1447

Principal Investigator: Duan Roth

Other Investigator: Keith C. Richardson

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.1 |
| Professional: | 1.1 |
| Others: | 0.0 |

Project Description:

Objectives: To determine the effect of autonomic ganglionectomy on the innervation of the albino rat iris.

Methods Employed: Superior cervical and ciliary ganglionectomy were performed on Osborn-Mendel rats. At variable post-operative intervals from two hours to one year, the irides were prepared by standard techniques of osmium tetroxide fixation and epoxy embedding. Thin sections were collected on two-hundred mesh or single-hole copper grids for viewing in the electron microscope. The iris stroma was surveyed from the ciliary processes to the pupillary sphincter.

Major Findings: Cervical sympathectomy produced ipsilateral ptosis and enophthalmos. Ciliary ganglionectomy effected a maximum pupil dilation which was immediate and sustained.

When the iris is examined two days to one year following sympathectomy no sign of neuronal degeneration is evident, nor is there a change in the stroma when compared to normal or control irides. However, degeneration appears in fine nerve processes of the stroma, especially along the dilator muscle,

as early as eighteen hours post-operatively. This phenomenon is prevalent at twenty-four hours and is completed by the second post-operative day. Although a few axons appear pale and swollen, most of the degenerating processes consist of electron dense axon debris, aggregated vesicular components, and mitochondrial fragments. As the mesaxon and axon membranes disappear the residual axon disintegrates within the substance of the Schwann cell cytoplasm. No increase in macrophage activity is apparent. Degenerating axons and normal axons may be situated side-by-side within the same extension of sheath cell cytoplasm.

Similar alterations are evident in degenerating unmyelinated axons after ciliary ganglionectomy and persist for several days. Myelinated fibers undergo Wallerian degeneration. After one week a mild atrophy of stromal cell processes accompanies the loss of normal nerve networks.

Significance: The concept prevails that ganglionectomy effects an irreversible morphological, pharmacological, and physiological degeneration in axon terminals. This tenet was supported by symptoms of ganglionectomy (ptosis, enophthalmos), alteration in staining potential (silver techniques), and disappearance of fluorescence in adrenergic nerve terminals.

The present work demonstrates for the first time the ultrastructural changes which characterize the morphology and time course of degeneration in the autonomic nervous system. Sympathetic denervation in the rat iris is more rapid and affects fewer fibers than ciliary ganglionectomy, although the morphology of neuronal degeneration is similar in both procedures.

Proposed Course of Project: The present work will be extended to include a description of permanganate-fixed irides after ciliary ganglionectomy, prior to publication. The project will then be terminated.

Honors and Awards: None

Publications: Roth, C. D. and Richardson, K. C.: Electron-microscopical studies on axonal degeneration in the rat iris following ganglionectomy. Am. J. Anat. (In press)

Serial No. NDB(I)-67 LNNS/NC 1448
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The innervation of the sphincter and dilator muscle of the iris

Previous Serial Number: LNS/NC 1448

Principal Investigator: Arthur I. Geltzer

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 1.0 |
| Others: | 0.0 |

Project Description:

Objectives: To study the fine structure of the innervation of the sphincter and dilator muscle of the iris.

Methods Employed: Kittens and young cats were subjected to unilateral and bilateral superior cervical ganglionectomies, unilateral ciliary ganglionectomies and combined bilateral superior cervical and unilateral ciliary ganglionectomy. The animals were sacrificed 20 hours to 6 months postoperatively. The unoperated side or in the case of bilateral experiments an unoperated animal served as the control.

The material was fixed by local perfusion through a cannula inserted into the posterior ciliary artery. The most informative fixation were obtained by local perfusion for 5 minutes with cold 1% $O_sO_4$ in a phosphate buffered solution bubbled with 95% $CO_2$ to bring the pH to 6.0. The local arterial perfusion was followed by direct perfusion of the anterior chamber for an additional 5 min. with the same fixative through a central incision in the cornea. The anterior segment was then removed "in situ"; the resulting cup of lens, iris and cornea were immersed in cold osmium and examined following the standard

electron microscope techniques of fixation and epoxy resin embedding.

Significance: From our observations it would appear that there are two morphologically distinct innervations to each of the muscles of the iris. The dilator muscle received an almost equal distribution of clear 500 A and granular (dense core) 500 A vesicular nerve terminals. The sphincter muscle is associated predominantly with nerve terminals having 500 A clear vesicles.

An identification of individual adrenergic and cholinergic nerve terminal without histochemical reaction has been observed. It is submitted that there exists a dual innervation of the feline dilator muscle to significant degree and to a lesser extent a dual innervation of the feline sphincter muscle. Selective degeneration experiments have been performed showing a rapid degeneration of the specific nerve terminals with sparing of the unaffected fibers.

Proposed Course of Project: The project is to be completed and findings published.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 LNNS/NC 1587
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: A blood-brain-barrier to peroxidase in the normal and injured brain of elasmobranchs

Previous Serial Number: None

Principal Investigator: Milton W. Brightman

Other Investigators: Igor Klatzo and Yngve Olsson

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.2 |
| Others: | 0.0 |

Project Description:

Objectives: To determine the fine structural basis for the blood-brain-barrier to peroxidase in the shark.

Methods Employed: Exogenous (horseradish) peroxidase is injected into the caudal vein or cerebral ventricles of nurse sharks and the ultimate distribution of the protein is detected electronmicroscopically by a cytochemical method.

Major Findings: The experiments have just been initiated.

Significance: In the shark brain, glial processes and end-feet radiate away from the vessel wall instead of ensheathing it. The cerebral ventricles are branched, forming many cerebellar diverticuli. Even after severe injury to the cerebral tissue, fluorescein-labelled proteins do not spread from the site of the wound. The investigation of these peculiarities by electron microscopic techniques may elucidate mechanisms of colloid passage that are fundamentally different from those in the mammalian brain.

Proposed Course of Project: To carry on, as outlined, the experiments which have been initiated.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 LNNS/NC 1588
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Some intercellular junctions in the vertebrate brain

Previous Serial Number: None

Principal Investigator: Milton W. Brightman

Other Investigator: Thomas S. Reese

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.7 |
| Others: | 0.0 |

Project Description:

Objectives: The objectives of the original study have been expanded to include the fine structural interrelations between ependymal and glial cells, those between the leptomeninges (incorporating a portion of project No. LNS/FN 1442), and between electrically coupled neurons in different species.

Methods Employed: Pieces of perfused-fixed mouse brains were stained, before embedment, with uranyl acetate in order to define the unit membranes. The tracer, horseradish peroxidase, (0.5 to 3.0 mg) and lanthanum hydroxide were injected into the cerebral ventricles of several mice whose brains were then fixed 30 min. later. The brains of additional specimens were fixed with glutaraldehyde dissolved in strongly hypertonic (1.5%, & 4%) sodium chloride solutions. The pial cells and underlying glial processes in these animals was also examined with peroxidase. In addition, junctions between electrically coupled neurons in the chicken and goldfish were studied. Some of these junctions were further analyzed with lanthanum hydroxide tracer.

Significance: The parenchymal extracellular space is patent throughout its extent except at certain endothelial and epithelial sites. The presumed loci of electrical coupling

between neurons in the fish and bird are not intermembranous fusions but are also patent. These junctional slits may be bridged by subunits which may subserve a cell-to-cell pathway for the diffusion of ions and current.

Proposed Course of Project: This more comprehensive account of intercellular junctions in the vertebrate brain is being prepared for publication. Project is completed.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-60 LNNS/FN 712
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The ascending and descending auditory connections in the primates.

Previous Serial Number: LNS/FN 712

Principal Investigator: Grant L. Rasmussen

Other Investigators: Prof. Jean Desmedt, Universite Libre de Bruxelles, Brussels, Belgium

Cooperating Units: Universite Libre de Bruxelles, Brussels, Belgium

Man Years

| | |
|---|---|
| Total: | 0 |
| Professional: | 0 |
| Other: | 0 |

Project Description:

Objectives: In general, to extend previous studies of the ascending and descending system of lower mammalian subjects to the primate which possess anatomical features more similar to that of man. In 1965 the specific objective was a study of auditory and related cerebral cortical regions that project cortiocofugally to the medial geniculate body, inferior colliculus and other areas of the thalamus and midbrain. This project was initiated in the laboratory of Prof. Jean Desmedt of the Universite Libre de Bruxelles in 1965 for the purpose of establishing an anatomical basis for follow-up physiological studies on the interaction of the higher set of ascending and descending conducting neurons in the process of hearing.

Methods Employed: Lesions of various sizes and different areas of the superior temporal gyrus and surrounding cortical regions were carefully removed by the suction method. Corticofugal degenerated fibers are being traced in serial sections stained by an axonal degeneration method to lower auditory centers and the relative population of fibers originating from the variously located cortical lesions is evaluated. Thirty cynomologus monkeys were used in this experiment.

Major Findings: All thirty monkey brains experimented upon in Brussels have been processed histologically but observations are too incomplete to give major findings at this time.

Significance: Anatomical knowledge of the cortical auditory connection in the monkey need be obtained in order to design appropriate physiological experiments for testing the functional role of the descending auditory system.

Proposed Course of Project: This project was inactive during the past year due to full time development in other investigations. Particularly, a new project concerning the existence of a regional vasomotor circuits regulating blood flow of the central auditory and visual systems. More time will be devoted to this project this year and possibly will resume a collaborative anatomico-physiological project on the primate auditory system with Dr. Desmedt.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-60 LNNS/FN 713
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: A study of the auditory afferent and efferent systems including the existence of regional vasomotor circuits of the central auditory and visual systems.

Previous Serial Number: LNS/FN 713

Principal Investigator: Grant L. Rasmussen

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.5 |
| Professional: | 0.7 |
| Other: | 0.8 |

Project Description:

Objectives: To continue to reveal unknown anatomical neuronal connections of the afferent and efferent divisions of the auditory system; to gain more information about the anatomical and functional interrelationships of these two systems. The immediate problems under study are:

1. To establish at the synaptic level the interconnections existing between the cochlear nuclei, superior olivary complex, the nuclei of the lateral lemniscus and the inferior colliculus as well as the auditory reflex connections with certain motor nuclei and the reticular formation.

2. To continue study at light microscope level of the morphological relationships of certain aberrant pathways and groups of fibers of the auditory and visual systems which appear to terminate about vessels supplying the nuclear masses of each system. The light microscope study is to be correlated by a separate electron microscopic investigation with Dr. Donald McDonald of this section.

Methods Employed: The newer and more effective techniques are chiefly relied upon for demonstration of axonal preterminal and terminal degeneration in the CNS subsequent to making lesions in appropriate regions. Also the Golgi and the histochemical method of Koelle, which we have modified to advantage, are heavily relied upon to furnish information not otherwise obtainable. The Richardson's silver method for demonstrating normal and degenerate unmyelinated fibers on cerebral vessels is employed as well as histochemical and fluorescence methods for demonstrating cholinergic and adrenergic neuronal element.

Major Findings: 1. No new major findings to report on efferent and afferent neuronal connections beyond those which were published this year in a book "Sensorineural Hearing Processes Disorders".

The collaborative project with Dr. Smith of Washington University, St. Louis, Missouri, has been concluded and the results previously reported appears this year in a book "Third Symposium on the Role of the Vestibular Organs in Space Exploration".

2. Anatomical evidence of a fine caliber system of fibers which may be concerned with regulation of localized blood-flow to different nuclear levels of the auditory and visual systems has been revealed with a modified Nauta technique in cat and the chinchilla. The fibers concerned originate and course varying distances in the classical well known ascending and descending auditory and visual pathways before departing external-wards to the glial-pial membrane and to become intimately associated with the penetrating vessels supplying the nuclei of each system. Some penetrate the glial-pial membrane but most fibers turn abruptly in reverse direction short of the glial membrane and accompany the intracerebral arteries and veins. The predispositions of these abberant coursing fibers for intimate association with intra cerebral vessels strongly indicates a vasomotor function. However, it has not been possible thus far to determine with light microscope methods whether or not these fibers actually terminate on the smooth muscle vasculature. Therefore, this important question is also being studied with the aid of techniques applicable to electron microscopy

Significance: 1. Knowledge of the neuronal relationships existing at the synaptic level between the afferent and efferent systems is basic to an understanding of the neuromechanisms of hearing. Such information is essential for a foundation upon which to design physiological experiments for testing the functional significance particularly of the descending conduction system.

2. The disclosure of a neurogenic mechanism for regulating regional blood flow of the auditory, visual and other systems of the C.N.S. would be important to physiologists studying the problem of localized blood flow of the brain.

Proposed Course of Project: 1. Study of the afferent and efferent auditory connections of the chinchilla and cat will be continued.

2. To continue investigation of intracerebral vasomotor neuronal connections with appropriate methods for light microscopy.

Honors and Awards: None

Publications:

Smith, Catherine A. and Rasmussen, G. L.: Nerve endings in the maculae and cristae of the chinchilla vestibule with a special reference to the efferents. In Graybiel, A. (Ed): Third Symposium on the Role of the Vestibular Organs in Space Exploration, published by NASA, 1968.

Rasmussen, G. L.: Efferent connections of the cochlear nucleus. In Graham, B.(Ed): Sensorineural Hearing Processes and Disorders. Little, Brown and Company Inc. 1967, pp 61-75

Serial No. NDB(I)-65 LNNS/FN 1229
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Fine structure of afferent and efferent nerve endings in the cochlear nucleus of normal and experimental animals.

Previous Serial Number: LNS/FN 1229

Principal Investigator: Thomas Reese

Other Investigators: Grant L. Rasmussen

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.4 |
| Professional: | 0.2 |
| Other: | 0.2 |

Project Description:

Objectives: To determine the ultrastructural difference between the cochlear nerve afferent endings in different subdivisions of the cochlear nucleus and those of efferent fibers originating from higher auditory centers as previously established by light microscopic studies of G. L. Rasmussen.

To study ultrastructural alterations in the nerve cells deprived of synapses (transynaptic degeneration).

Methods Employed: The electron microscopical studies will be correlated with experimental results obtained from light microscopic studies. The brain is to be perfused-fixed with an appropriate fixative to insure good material for electron microscopic study. The afferent type endings are to be identified by ultrastructural alterations resulting from destruction of a spiral ganglion in chinchilla and the time course of this degenerative process studied. The efferent type of endings are to be identified by ultrastructural alterations following destruction of the superior olive. Comparable areas of the cochlear nucleus of the unoperated side is used as a control.

Major Findings: Studies thus far on the normal anterior ventral nucleus reveal two distinct types of synapses based on size of vesicles. In experimental animals, an electronmicrographic study reveals that the typical calyciform endings of the cochlear nerve possessing the larger vesicles disappear while the remaining unaltered non-afferent synapses on the de-afferentiated cell possess only the smaller vesicles. A preliminary report appears in the Anatomical Record, vol. 153, p.408, 1966. During the course of this study it has been appreciated that the afferent endings in the cochlear nucleus undergo degeneration by swelling and lysis rather than by shrinkage (with or wothout neurofibrillar formation) followed by phagocytosis of the axonal fragemnt by glia, a sequence of events that had come to be accepted as the only pattern of synaptic degeneration within the central nervous system. Studies on other auditory nuclei are too incomplete to furnish any major findings at this time.

Significance: This study provides much needed information concerning ultrastructural alterations that accompany deafferentiation of nerve cells of the brain and particularly the identification of different functional types of nerve endings on the basis of ultrastructural dissimiliarities. The discovery of a new type of degeneration in axonal endings separated from their cell bodies will lead to alternative interpretations of some experimental neuroanatomical studies which have depended on recognition of degenerating endings with the electron microscope.

Proposed Course of Project: This project received little attention during the past year and will be continued more actively during this year and will include the synaptology, as revealed with the electron microscope, of other subdivisions of the cochlear nuclei.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 LNNS/FN 1231
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The fine structure of the olfactory bulb.

Previous Serial Number: LNS/FN 1231

Principal Investigator: Thomas Reese

Other Investigators: Milton W. Brightman

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Others: | 0.0 |

Project Description:

Objectives: To study the fine structure of the olfactory bulb in the rat.

Methods Employed: Olfactory bulbs were fixed with buffered osmium tetroxide and glutaraldehyde by vascular perfusion. Thin sections were examined by light and electron microscopy.

Major Findings: A hitherto undescribed synapse, viz, dendro-dendritic, has been found in both the olfactory glomerulus and the external plexiform layer. The origins of both the pre- and post-synaptic components of the dendro-dendritic synapses in the external plexiform have been identified by studying serial electron micrographs. By this identification, in conjunction with concurrent physiological studies, it is shown that the dendro-dendritic synapses serve as a pathway for recurrent and lateral inhibition in the external plexiform layer of the olfactory bulb; this finding is the subject of the report listed below*. The dendro-dendritic synapses in the olfactory glomeruli probably serve quite a different function, which is now being studied. Around the olfactory glomeruli is a system of calyceal envelopments of one small granule neuron by its neighbor, reminiscent of the calyces in the vestibular apparatus.

* Dendrodendritic synaptic pathway for inhibition in the olfactory bulb, Exp. Neurol., 1966, 14, 44-56

Significance: Our observations have already provided a morphological basis for such phenomena as lateral and recurrent inhibition in the olfactory bulb. It is anticipated, that similar synaptic mechanisms will be found in other sensory systems, particularly in the retina where similar synaptic arrangements have already been reported.

Proposed Course of Project: A preliminary report on the glomerular dendro-dendritic synapses has been published and a full report has been prepared which includes some new data on the origins of the dendro-dendritic synapses obtained by combining the Golgi technique with electron microscopy. The completed report on this work will be sent to the American Journal of Anatomy. It is anticipated that another report based on serial sectioning of synapses in the olfactory bulb will be finished in the coming year, bringing this project to a close.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-67 LNNS/FN 1442
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Fine structural study of the movements of protein within the cerebral meninges, the median eminence and area postrema.

Previous Serial Number: LNS/FN 1442

Principal Investigator : T. S. Reese

Other Investigators: M. W. Brightman

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 0.5 |
| Other" | 0.5 |

Project Description:

Objectives: To determine the pathways used by substances passing from the cerebrospinal fluid through the meninges into the parenchyma of the brain and into veins and other tissues surrounding the brain. This approach has now been expanded to include the passage of substances between ventricular and subarachnoid cerebrospinal fluid and specialized areas of the brain such as the median eminence and area postrema.

Methods Employed: The cytochemical method of Karnovsky is used to localize purified horseradish peroxidase after it is injected into the cerebral spinal fluid of living, anesthetized animals.

Major Findings: Peroxidase injected into the cerebrospinal fluid of the subarachnoid space in the mouse is able to pass between cells of the pia-arachnoid layer, cross the basement lamina of the brain, and pass between the astrocytic processes forming the limiting surface of the brain to reach the generally open interstitial spaces of the brain. However, peroxidase does not cross from ventricular cerebrospinal fluid into the median eminence and area postrema. The basis of this selective barrier is a system of "tight" junctions within the specialized epithelial cells lining the ventricular surface over these regions.

Significance: Since open channels are available for exchange of substances between spinal fluid and brain across its pial-glial surface, this exchange would necessarily have a significant passive and nonselective component. However, any exchange between median eminence or area postrema and ventricular cerebrospinal fluid would be limited by the permeabilities of the covering epithelial cells. This epithelium could then serve as a secondary blood-brain barrier system in these regions which lack the typical blood-brain barrier situated at the capillary endothelium. The presence of this "barrier" type of epithelium might also be the reason why neurosecretory hormones released into the median eminence enter the hypophyseal portal system rather than escaping into the cerebrospinal fluid.

Proposed Course of Project: The first finding described above will be included in a comprehensive article on extracellular space and junctions in the brain by M. W. Brightman and T. S. Reese (LNNS/NC 1444). The localization of barriers to the intercellular movement of peroxidase at the dural-arachnoid membrane has not been completed. The work on the median eminence and area postrema has been reported in a preliminary note (Anat. Rec., 1968) and, it is expected, will be completed and fully reported in the next year.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-68 LNNS/FN 1589
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The fine structure of the innervation of cerebral blood vessels, with respect to the regional control of the cerebral circulation.

Previous Serial Number: None

Principal Investigator: Donald M. McDonald

Other Investigators: Grant L. Rasmussen

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.7 |
| Professional: | 1.2 |
| Other: | 0.5 |

Project Description:

Objectives: To study the ultrastructural relationship of the vasomotor nerve fibers and their endings to the extra- and intracerebral blood vessels of cat and chinchilla.

To investigate particularly the manner of innervation of the penetrating vessels at the ultrastructural level in order to obtain anatomical evidence for differential regional blood flow of the brain indicated by recent physiological studies.

Methods Employed: Appropriate perfusion fixatives and histochemical methods for light and electronmicroscopical study will be used on normal and experimental animals; to study degenerating nerves and their terminals following lesions in different regions of the auditory and visual systems. Control experiments will also be carried out by eliminating the better known sympathetic and afferent innervation of the non-penetrating cerebral vessels, which will involve complete cervical sympathectomy and the cutting of certain cranial nerves.

Major Findings: The electronmicroscopy findings thus far support the recent observations in Nauta preparations (GLR) that certain superficially coursing fibers originating from all levels of the auditory system direct themselves toward penetrating vessels supplying auditory nuclei. One region in particular studied thus far is near the merging abducens nerves where fine myelinated nerve fibers originating from the inferior colliculus tend to accumulate about penetrating vessels supplying the superior olivary complex. In normal material a study of the ultrastructure of the surface of the brain in this area has shown in addition to numerous small myelinated fibers, myriads of unmyelinated axons, related intimately to penetrating vessels. Electron microscopy studies on experimental material is too incomplete at this time to make definite conclusions regarding the ultimate termination of the fibers in question.

Significance: Anatomical information revealing a neurogenic vasomotor mechanism for regulation of regional blood-flow in different systems such as auditory and visual would be of immediate importance to physiologists studying the problem of differential cerebral circulation.

Proposed Course of Project: This will be a continuing project.

Honors and Awards: None

Publications: None

Annual Report of the
Laboratory of Neurophysiology, Intramural Research
National Institute of Neurological Diseases and Blindness

July 1, 1967 through June 30, 1968

M.G.F. Fuortes, M.D., Chief

The Laboratory of Neurophysiology, NINDB, is a new unit, established in January 1968 as part of the reorganization planned in connection with the new Building 36. The personnel of the Laboratory was previously part of the Spinal Cord Section of the Laboratory of Neurophysiology, NIMH, and all projects described in the present report had been started before the reorganization of the Laboratory. Taking these projects as a group, their overall objective is the understanding of 1) the properties of individual nerve cells and of 2) the mechanisms governing the relations between nerve cells.

We know that nerve cells collect information from the environment, encode it in messages which can be transmitted at a distance, correlate information from different sources, simplify complex patterns by extracting the features which are most important for given purposes (e.g. for survival), store information and send integrated messages to different organs, thus controlling their functions. In order to understand how nerve cells perform these activities, it is essential to investigate the basic organization of the nervous system as well as the processes of reception, generation and conduction of nerve impulses, transmission across synapses and memory. Other features of nerve cells are important and should be investigated: study of the properties mentioned above, however, already involves more problems than one may hope to solve in many years of work.

It has become clear in recent years that two types of membranes play essential roles in the functions of nerve cells: membranes such as those of peripheral axons which have the properties required for conduction of all-or-none nerve impulses and membranes of receptors and of pre and postsynaptic structures, which are characterized by other properties. None of the projects carried out in the Laboratory of Neurophysiology is concerned with conduction of impulses: this field is considerably more advanced than other areas of general neurophysiology, has become highly specialized and is actively investigated in the Laboratory of Biophysics, NINDB, as well as in the Laboratory of Neurobiology, NIMH. Our own projects have been confined to studies of receptors, synapses and functions of nerve cells in the central nervous system.

Summary con't.

The problem of reception - how nerve cells are activated by the environment - has been studied in collaboration with the Massachusetts Institute of Technology. The aim of their research is to determine how light produces electrical changes in photoreceptor cells. Experiments performed on cells of the ventral eye of Limulus, indicate that the membrane of these cells has complicated electrical properties. In darkness and in the absence of currents, the membrane has moderate resistance. The resistance decreases when currents of moderate intensity flow from inside to outside (depolarizing currents) and increases drastically with stronger currents in the same direction or with currents in the opposite direction. Light produces the same changes as moderate depolarizing currents: it decreases membrane potential and membrane resistance. These findings suggest that light has the same effects as a current-source while a number of other results may be interpreted assuming that light affects the active transport of ions across the membrane of photoreceptor cells. Whether or not this interpretation is valid, it seems clear that these experiments have revealed some new and important properties of photoreceptors.

Studies on synaptic organization have been performed by Dr. R.E. Burke who is now in the Department of Physiology of the University of Goteborg, Sweden, where he works in collaboration with Prof. A. Lundberg. They find that motoneurons in the spinal cord of cats divide in two groups: "F" motoneurons innervating muscle units producing fast twitches and "S" motoneurons, innervating units which produce slow twitches. "F" motoneurons develop short-lasting synaptic potentials which do not sum effectively in time. As a consequence of these properties the firing evoked by sustained inpingement shows rapid adaptation. "S" motoneurons, by contrast, develop longer lasting synaptic potentials, are subject to effective temporal summation and discharge slowly-adapting trains of impulses following prolonged impingement. It is presumed that these different properties of the two types of motoneurons depend in part upon the different localization of the synaptic endings, which may be more concentrated around the soma in "F" motoneurons and may extend farther in the periphery of the dendritic tree, in "S" motoneurons.

It has been observed, however, that "F" and "S" motoneurons show respectively fast and slow accommodation also when they are stimulated directly by extrinsic currents. It must be concluded, therefore, that the different properties of their impulse discharges are due in part to specific properties of their membranes.

Summary con't.

The observation that correlations exist between the properties of motoneurons and those of the motor units they innervate raises the possibility that motoneurons exert some control upon the properties of muscle fibers. In support of this notion it was found that the properties of muscle fiber are changed whenever the flow of impulses along their motor nerves is chronically altered. Conversely, if the muscle is relieved of all load without altering the nerve discharges, the contractile properties of the muscle fibers remain normal.

Thus, the control of muscle properties is closely associated to the nerve impulses reaching the muscle from the motor nerve. These findings are an example of the control exerted by nerve cells upon effector organs over long periods of time and may provide a starting point for studies on plasticity, learning and memory.

Serial No.: NDB(I)-58 LNP/OC-501
1. Laboratory of Neurophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Generation of impulses in nerve cells

Previous Serial Number: NDB(I)-58 LNP/SC-501

Principal Investigators: Phillip G. Nelson, M.D.
Robert E. Burke, M.D.

Other Investigators: David J. Mishelevich, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 2.2 |
| Professional: | 0.7 |
| Other: | 1.5 |

Project Description:

Objectives: To measure the process of accommodation in motoneurons and to correlate this with the properties of the muscle units innervated by the motoneurons.

Methods Employed: Intracellular recordings from cat spinal motoneurons were obtained by conventional means. The motoneurons were stimulated by currents passed through the recording pipettes. Accommodative properties of the nerve cells were measured by passing linearly increasing currents across the cell membrane. Muscle unit twitch characteristics were measured by a strain gauge attached to the appropriate tendons.

Major Findings: Motoneurons can be divided into one group which shows negligible accommodation and another group that shows moderate to marked accommodation. Those motoneurons that do not accommodate innervate muscle units with low twitch amplitude and relatively slow interactions. Motoneurons that do accommodate to linearly rising currents innervate muscle units with relatively large twitch amplitudes and with fast contractions.

Significance to bio-medical research and the program of the Institute: The distinction between tonic and phasic systems and between fast and slow systems seems to be fundamental in the

nervous system and in the neuromuscular system in particular. The work on accommodation would seem to indicate that the membrane properties of motoneurons themselves partially determine the tendency of different cells to respond differentially to steady or rapidly changing stimuli. The motoneurons connected to small slow twitch muscle units are those most easily excited by slowly changing inputs.

Proposed Course of the Project: Experiments may be done in which attempts will be made to characterize adaptive processes in motoneurons by studying the firing rate as function of time following intracellular application of steps of depolarizing currents. This adaptation will be correlated with the contractile properties of the muscle units innervated by the different motoneurons.

Honors and Awards: None

Publications: None

Serial No.:NDB(I)-62 LNP/OC-934
1. Laboratory of Neurophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Basic Mechanisms of Synaptic Transmission

**Previous Serial Number:** NDB(I)-62 LNP/SC-934

**Principal Investigator:** Robert E. Burke

**Other Investigators:** Anders Lundberg[1/]

**Cooperating Units:** 1/Department of Physiology
University of Goteborg
Goteborg SV, Sweden

**Man Years:**

| | |
|---|---|
| Total: | 2.5 |
| Professional: | 1.0 |
| Other: | 1.5 |

**Project Description:**

Objectives: During the past year, this project has continued to involve study of motoneuronal properties of the amount and organization of synaptic input to the cell from specified sources and of the properties of the muscle fibers innervated by a given motoneuron. The system used for investigation of this question has been the lumbosacral extensor motor units of the cat plus the group Ia afferent input to such units. The basic objective in this work has been an understanding of the mechanisms by which cells with complex geometry integrate synaptic information from a variety of sources to produce a patterned train of action potentials.

Methods employed: Conventional electrophysiologic techniques of intracellular recording has been used. In addition, methods have been used permitting measurement of muscle mechanical activity during intracellular recording without dislodgement of the microelectrode.

Major findings: The summation of unitary post-synaptic potentials in motoneurons to produce firing of the cells is different for "F" motoneurons (those innervating fast twitch muscle units) as

compared with "S" motoneurons (those innervating slow muscle units). Summation is more effective for the "S" units and repetitive firing is easier to elicit in these cells. The observations are consistent with a tonic behavior of the "S" motor units and a phasic behavior for the "F" motor units, and appears related to the localization of the synaptic input to the cells on the dendritic trees of the two cell types.

Significance to bio-medical research and the program of the Institute: The different behavior of the Type F and S motor units is related to general properties of the neuromuscular system. Large, fast twitch motor units appear to be less affected by group Ia afferent input than are small slow twitch motor units. By inference other perhaps supraspinal sources may have greater dominance in the Type F motor units.

Proposed course of the project: Further studies on dendritic location of synapses will be conducted utilizing the recurrent inhibitory system in the spinal cord.

Honors and Awards: None

Publications:

Burke, R.E.: Motor Unit Types of Cat Triceps Surae Muscle, J. Physiol. 193: 141-160, 1967.

Burke, R.E.: Composite Nature of the Monosynaptic Excitatory Postsynaptic Potential, J. Neurophysiol. 30: 1114-1137, 1967.

Nelson, P.G. and Frank, K.: Anomalous Rectification in Cat Spinal Motoneurons and Effect of Polarizing Currents on Excitatory Postsynaptic Potential, J. Neurophysiol. 30: 1097-1113, 1967.

Rall, W., Burke, R.E., Smith, T.G., Nelson, P.G. and Frank, K.: Dendritic Location of Synapses and Possible Mechanisms for the Monosynaptic EPSP in Motoneurons, J. Neurophysiol. 30: 1169-1193.

Serial No.:NDB(I)-62 LNP/OC-973
1. Laboratory of Neurophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Integrative mechanisms in the central auditory pathway

Previous Serial Number: NDB(I)-62 LNP/SC-973

Principal Investigator: Phillip G. Nelson

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0 |
| Professional: | 0 |
| Other: | 0 |

Project Description:

This project has been inactive during the past year but will be activated during Fiscal Year 1969.

Proposed course of project: Recordings from single units in the central auditory pathway of unanesthetized chronically prepared squirrel monkeys will be performed. Further theoretical work and modeling of the auditory system is contemplated.

Honors and Awards: None

Publications: None

Serial No.:NDB(I)-65 LNP/OC-1239
1. Laboratory of Neurophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Photoreceptors in the Limulus eyes

Previous Serial Number: NDB(I)-65 LNP/SC-1239

Principal Investigator: Thomas G. Smith, Jr.

Other Investigators: Joel E. Brown 1/
William K. Stell

Cooperating Units: 1/ Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts

Man Years:

| | |
|---|---|
| Total: | 3 |
| Professional: | 2.5 |
| Other: | 0.5 |

Project Description:

Objectives: The objective of this research is to gain insight into the means by which light is transduced to an electrical current in photoreceptors. The intracellularly recorded potentials of the photoreceptor cells of the horseshoe crab, Limulus polyphemus, are the object of study.

Methods employed: The responses of the photoreceptor cells to light and electrical stimulation are analyzed under a variety of experimental conditions. In this way, the role of visual pigments of the photoreceptor cell membrane and of the various ions in photoreception can be studied.

Major findings: The major result of the past year has been characterization of some of the mechanisms underlying the generator potential which can be recorded with intracellular microelectrodes from the photoreceptor cells of Limulus eyes when they are stimulated with light. In the first place, the generator potential has been shown not, as commonly thought, to be the result of a conductance increase in the photoreceptor cell membrane. Rather the experimental results have led to an alternate hypothesis. This hypothesis is that the photoreceptor membrane has an

electrogenic sodium pump and that the light-induced generator potential is a consequence of an alteration of the electrogenic component of this pump.

Significance to bio-medical research and the program of the Institute: The experimental results and the attendant hypothesis have given further emphasis to the primary role of the photoreceptor membrane in the transducer mechanisms in visual processes. In addition, they narrow the hiatus in our knowledge of the steps involved between the absorption of light by rhodopsin and the electrical signals generated in photoreceptors. Thus, the main question of the transducer action in visual processes becomes the question of how the rhodopsin molecules, located in the photoreceptor membrane, affect those nearby membrane molecules that make up the sodium pump machinery.

Proposed course of project: It is proposed to continue the study of the generator potential and the electrogenic sodium pump in Limulus photoreceptors by undertaking experiments which will test the hypothesis that alterations in sodium pump activity result in the generator potential. In addition, it is proposed to develop techniques which will allow simultaneous and independent measurement of the rhodopsin content of the cell by microspectrophotometry and of the cell's visual responses by electrophysiology. Such techniques may give insight into how rhodopsin may regulate the sodium pump.

Honors and Awards: None

Publications:

Brown, J.E., Murray, J. R. and Smith, T.G.: Photoelectric Potential from Photoreceptor Cells in Ventral Eye of Limulus. Science, 158: 665-666, 1967.

Serial No.:NDB(I)-67 LNP/OC-1456
1. Laboratory of Neurophysiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Trophic Mechanisms in the Nervous System

Previous Serial Number: NDB(I)-67 LNP/SC-1456

Principal Investigators: Norman Robbins
Gerald D. Fischbach
Phillip G. Nelson

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 3.3 |
| Professional: | 2.3 |
| Other: | 1.0 |

Project Description:

Objectives: 1. From cross-innervation studies, it is known that the motoneuron in some way controls the contractile properties of the muscle fibers it innervates. The control is dependent on some neurohumor released by and intrinsic to different motoneurones, or it is dependent on neural and muscular activity. We devised experiments to distinguish between these possibilities. 2. We have also carried out a cross-innervation experiment to define the nature of the change in the population of cross-innervated fibers, and to relate this to histochemical findings in the same muscle. 3. As demonstrated by studies of denervated muscle, the motor nerve also regulates various properties of the muscle membrane, such as acetylcholine sensitivity and membrane resistance. To date, it has been difficult to separate the multiple effects of denervation from the accompanying effects of synaptic disuse. We have studied a preparation in which profound and chronic disuse has produced and been documented; we wished to determine which features of the post-synaptic structure are subject to modification by virtue of disuse alone. 4. The same preparation is also being used to provide a systematic description of the effects of disuse on synaptic function in general. As opposed to the synapses in the C.N.S., the neuromuscular junction is relatively well understood and uncomplicated, and has a

known transmitter. Presynaptic and postsynaptic changes can be separately evaluated and transmitter release can be directly measured. This will be the first intensive study of a disused synapse, where the disuse has been obtained without the complications of trauma or transneuronal effects.

Methods employed: In vitro chamber with strain-gauge to measure isometric contractile properties of muscle. Standard microelectrode assembly for in vitro study of intracellular responses of muscle.

Major findings: 1. We measured several isometric contractile properties of rat soleus muscle which had been subject to disuse for 4 weeks. The muscles showed a significant "speeding" by every parameter measured. On the basis of a previous EMG study (see last year's annual report), it was concluded that the changes in contractile properties must be due to a change in either pattern of neural firing or aggregate number of impulses. This implies that adult muscle is continually subject to modification of contractile property, depending on the pattern of neural activation it receives. 2. A parallel study in tenotomized cat muscle gave results consistent with the above hypothesis. An EMG study of cat soleus revealed no gross change in activity for periods of weeks after tenotomy. As expected, no significant changes in contractile properties were observed in solei after chronic tenotomy, despite the presence of marked atrophy. 3. The acetylcholine-sensitive region of normal muscle is restricted to a region about 150μ on either side of the end-plate. After imposition of neuromuscular disuse, the region of sensitivity was found to enlarge, reaching a maximum about 8-10 days after onset of disuse, and returning to normal in weeks thereafter. As opposed to denervation, then, the acetylcholine sensitivity of the intact but disused synapse responds only transiently to disuse. At no time in this course of events were changes in frequency of miniature end-plate potentials found; only the decrease in acetylcholine released with nerve impulses can be implicated. Finally, despite the increased region of acetycholine sensitivity observed, the size of miniature end-plate potentials (an index of the amount of quantal release of acetylcholine) did not show a significant change in size when normalized for change in input resistance. Therefore, the spread of acetylcholine sensitivity observed probably has little physiologic significance with regard to postsynaptic sensitivity in the process of synaptic transmission. Other aspects of synaptic transmission, however, remain to be studied (v.i.).

Significance to bio-medical research and the program of the Institute: A study of the effect of chronic use and disuse on muscle contractile properties and on neuromuscular transmission is an important step toward the understanding of the trophic relations between nerve and muscle. In addition, the results may have bearing on synaptic plasticity in the C.N.S. Modifiability of intercellular connections may, in turn, be the basis of learning and memory considered in the broad sense. It may also lead to new approaches to the understanding and treatment of various chronic neurological diseases.

Documentation of the degree of synaptic activation may resolve the confusion that has been generated by studies in which use or disuse were assumed but not measured.

Proposed course of study: 1. Measurement of the electrophysiological properties of disused and excessively used neuromuscular junctions. Presynaptic function, i.e. transmitter depletion and mobilization during repetitive stimulation remains to be studied at disused junctions. In excessively used muscle, the effects of repetitive stimulation of used and disused muscles will be measured by bio-assay. Choline acetylase activity and uptake by nerve of acetylcholine precursers may be measured. 3. The morphology of used and disused end-plates will be studied with a silver impregnation technique. 4. The correlation between muscle contractile speed and histochemical type will be pursued by studying these properties in single fibers. 5. As part of a search for the best system in which to study the long term interaction between excitable cells, muscle cells in tissue culture will be studied. Emphasis will be placed on the developmental aspect of membrane properties and on intercellular communication. A long range goal is to follow the evolution of nerve-muscle contact and to study the effect on this evolution of various patterns of electrical stimulation. 5. A more detailed description of motor unit firing patterns including auto and cross-correlation analyses will be undertaken.

Honors and Awards: None

Publications:

Fischbach, Gerald and Robbins, Norman.: Changes in Contractile Properties of Disused Muscle. The Physiologist, 10: 170, 1967.

ANNUAL REPORT
July 1, 1967 through June 30, 1968
Laboratory of Biophysics, Intramural Research
National Institute of Neurological Diseases and Blindness

Robert E Taylor, Acting Chief

Membranes, Ions and Impulses is the title of a 569 page book that was written by Dr. K. S. Cole, with the support of the laboratory and of the University of California, Berkeley, and was published by the University of California Press in June 1968 under a sub-title "A Chapter of Classical Biophysics." It traces the development of the understanding of the electrical properties of cell membranes with which the author has been associated, from the discovery of the membrane capacity to the quantitative description of the membrane ion conductances and their role in nerve impulse conduction. Some of the more recent work is reviewed as an introduction to the problems of mechanisms which lie ahead and, when solved, will be a chapter of molecular biophysics.

The Head, Section on Mathematical Biophysics has completed a pioneering movie, "Impulse Propagation in a Nerve Fiber" which utilizes the computer obtained solutions of a simplified set of non-linear differential equations. Nerve impulse initiation and propagation is demonstrated and animation and sound were added. The film lasts twenty minutes and is available for loan from the Acquisition, Retention and Distribution Center, National Medical Audiovisual Center, Atlanta, Georgia 30333 as well as from the Laboratory of Biophysics for local use.

Work is continuing on a number of problems, most of which are closely related to the properties of cell membranes. Membranes are implicated not only in electrical activity and the transfer of materials into and out of cells but also in macromolecular synthesis, enzyme activity, the release and effects of hormones, immunological phenomena and probably in the initiation of antibody production. Studies have been made in Woods Hole and in Chile using squid giant axons where it is possible to replace the internal contents with known solutions. In experiments with low external calcium and high potassium it has been estimated that the outer surface of the membrane contains negative fixed charges every 8.5 angstroms in the vicinity of the regions responsible for the sodium and potassium ion movements. Further experimental work has been done with ammonium and other ions, which substitute partially for both sodium and potassium and with heavy water which appears to effect the time constant for the turning on of the sodium conductance mechanism. It is felt that the effects of heavy water support the suggestion that protonic conduction through lattice defects

in some hydrogen bonded structure in the membrane is involved in the control mechanisms for the conductance for sodium ions.

A system has been devised for data taken on 35 mm film to be read, analyzed and plotted by the LINC computer or read by the LINC and analyzed in greater detail by the central NIH computer.

Myxicola infundibulum, a sand worm, can now be obtained from Canada at any time, keeps well in a small aquarium, and supplies a central nerve cord which is now being routinely used for voltage clamping studies. There seems to be no reason why this nerve cannot be perfused in the same way as the squid giant axon and should be suitable for a number of biochemical studies in addition to the now classical electrophysiological approach.

Steady progress is evident on theoretical studies of noise fluctuations of membrane potential in nerve, on the utilization of recently developed theories of optimization for the study of nervous control of muscular movement and on the construction and use of artificial lipid bilayers. Projects using the newly acquired electron paramagnetic resonance apparatus are being planned and these will be begun when the necessary auxiliary components are obtained.

The original Chief of the laboratory was presented with the National Medal of Science on February 13, 1968 by the President of the United States.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Ionic Permeabilities of Excitable Membranes. Electrical Experiments and Analyses.

Previous Serial Number: Same

Principal Investigator: Cole, Kenneth S., and Binstock, L.

Other Investigators: Goldman, L. (University of Maryland)

Cooperating Units: University of California, Berkeley, California
University of Maryland, College Park, Md.

Man Years

| | |
|---|---|
| Total: | 2.6 |
| Professional: | 1.5 |
| Other: | 1.1 |

Project Description:

Objectives: The ionic current flow across the membrane of the squid giant axon and the nerve cord of Myxicola have been measured, without the complications of excitation and propagation, after a sudden change of the membrane potential. The currents have been analyzed in terms of the membrane permeability to sodium and potassium ions. Much of classical nerve physiology is explained by these permeabilities which are themselves not understood. The long range objectives are the further interpretation of nerve function in terms of these fast ionic permeabilities and the elucidation of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Measurements of the ionic current flow across the membrane of the giant axon of the squid and the nerve cord of Myxicola, without the complications of excitation and propagation have been improved by the simultaneous use of replacement of internal contents and continuous perfusion of known solutions.

Major Findings: The development of our understanding of the ionic movements in relation to the electrical activity in nerve membranes has been critically and extensively

reviewed in a forthcoming volume on Membranes, Ions and Impulses.

Myxicola infundibulum, a marine burrowing sand worm is now available in the Laboratory on a regular basis all year-round. The dissection of the single giant axon (450 to 900 microns) from the nerve cord is no longer so tedious. Normal values for resting and action potentials are 70 millivolts (measured either with impaled micropipettes at University of Maryland or coaxial electrodes at NIH) and 91 millivolts (measured with coaxial electrodes), respectively.

The Myxicola giant axon preparation survives voltage clamping for several hours with many solution changes. Under space-voltage clamp it exhibits current-voltage relations similar to those described for the squid axon. The early inward current component is reduced in a solution with low sodium concentration. The resting membrane potential is determined chiefly by potassium.

Scientific Significance: A thorough understanding of the relationships between ionic concentrations, inside and outside of the cell and the dependence of the ionic currents on potential are necessary to understand the normal functioning of the excitable system as part of the attempts to elucidate the molecular structure of the membrane.

Proposed Course of Project: Collaborative projects with the University of California at Berkeley and the University of Maryland will continue.

The Myxicola giant axon is similar in all its essential features to the squid giant axon and it survives space-voltage clamping very well. The next step of achieving a satisfactory simultaneous internal perfusion and voltage clamping of the axon from Myxicola is being vigorously attacked and is expected to be solved. This will make it possible to do experiments in Bethesda which are now possible only during field trips.

Honors and Awards:

Kenneth S. Cole 1) Silver Medallion on 200th Anniversery of College of Physicians and Surgeons, Columbia University, October 1967.
2) National Medal of Science, February 1968.

Publications:

Binstock, L., and Goldman, L.: Giant axon of Myxicola: Some membrane properties as observed under voltage clamp. Science 158: 1467-1469, December 1967.

Goldman, L.: The effects of some ion on the membrane potential of the giant axon of Myxicola. J. of Cell. Physiol. (in press).

Cole, K. S.: Membrane watching. J. Gen. Physiol. 51: 4(2), 1s-7s, May 1968.

Cole, K. S.: Membranes, Ions and Impulses. Berkeley, University of California Press, 1968, 569 pp.

Serial No. NDB(I)-62 LB/CB 939
1. Biophysics
2. Cellular Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses with Alterations of Environments.

**Previous Serial Number:** Same

**Principal Investigator:** Gilbert, D. L.

**Other Investigators:** Ehrenstein, G. M., Lecar, H., Taylor, R. E, Binstock, L., Rojas, E. and Atwater, I. (Universidad de Chile)

**Cooperating Units:** Marine Biological Laboratory, Woods Hole, Massachusetts
Laboratorio de Biologia Celular, Montemar, Chile

**Man Years**

| | |
|---|---|
| Total: | 2.9 |
| Professional: | 2.5 |
| Other: | 0.4 |

**Project Description:**

Objectives: To study the ionic current flow across the nerve membrane without the complications of excitation and propagation in terms of individual ion currents and to observe the effects of changes in normal internal and external ionic concentrations. The long range objectives are the interpretation of nerve function in terms of ion movements and the elucidation of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard voltage clamp techniques are employed with the addition of a control of the internal environment by replacement and perfusion.

Major Findings: The effect of 10 mM potassium ferricyanide on the electrical properties of the squid axon has been investigated. This redox agent diminishes the amplitude

of the action potential and depolarizes the membrane potential by about 20 mV. When the axon was voltage-clamped, using a hyperpolarizing prepulse, the currents of both potassium and sodium were little affected, if at all. The leakage current was increased by this agent. A possible explanation of this effect is that the permeability of some ion in the leakage channel is increased. However, by monitering the potential on an internal platinum wire for hours, there was no change, indicating that the ferricyanide ion did not permeate. Also, there was no indication of a yellow color, which could also be indicative of the ferricyanide ion, in the axon. The action does not seem to be redox in mechanism since potassium ferrocyanide seems to have a similar action. The tentative conclusion is therefore that the substance increases the permeability of some ion excluding the ferricyanide ion.

The effect of calcium on the steady state potassium currents measured under voltage-clamped conditions has also been studied. A model has been chosen which is consistent with our experimental findings. Upon analysis of this model, we conclude that on the outer membrane surface fixed negative charges exist in the vicinity of the sodium and potassium mechanisms for carrying these ions across the membrane during an action potential. The average distance between the negative charges is 8.5 $A^{o}$. Addition of calcium and/or magnesium combines with these negative charges and neutralizes them.

Further observations have been made on the progressive changes which occur in the membrane of the squid axon in the absence of calcium. One effect has been found which tended to obscure the interpretation of earlier data, viz., the leakage conductance which is normally cation selective is joined by a component in which the chloride conductance is high.

Scientific Significance: Redox substances have previously been shown to influence the electrical properties of nerves. However, in our present study, it appears that perhaps some of the effects of redox substances might not be necessarily due to the redox potential, per se.

Recently, other investigators using different methods, have concluded that there are fixed negative charges on the outer surface of the membrane. Our studies give an estimate of the density of these charges.

Chandler, Hodgkin and Meves previously gave an estimate of the fixed negative charges on the inner surface of the membrane at low internal ionic strength. Control of these charges by changing the ionic environment could possibly provide an explanation of ionic antagonism.

Proposed Course of Project: Only by continued and extensive investigation of the effects of changes in internal and external ion concentrations will the evidence be forthcoming which can provide the basis of a rational attack on the problem of molecular mechanisms in the membrane. These experiments will be conducted on the axons of the squid obtained in Woods Hole, Massachusetts and in Chile.

Honors and Awards: None

Publications:

Lecar, H., Ehrenstein, G., Binstock, L., and Taylor, R. E: Removal of potassium negative resistance in perfused squid giant axons. J. Gen. Physiol. 50: 1499-1515, July 1967.

Taylor, R. E: The role of inorganic ions in the nerve impulse. In Quarton, Gardner C., Melnechuk, Theodore and Schmitt, Francis O. (Eds.): The Neurosciences. New York, N.Y., Rockefeller University Press, 1968, pp. 305-312.

Gilbert, D. L.: The interdependence between the biosphere and the atmosphere. Resp. Physiol. (In Press).

Serial No. NDB(I)-62 LB/I 940
1. Biophysics
2. Instrumentation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Ionic Permeabilities of the Squid Giant Axon. Effects of Chemical Agents.

**Previous Serial Number:** Same

**Principal Investigators:** Binstock, L., Lecar, H. and Stillman, I.

**Other Investigators:** None

**Cooperating Unit:** Marine Biological Laboratory, Woods Hole, Massachusetts

**Man Years**

| | |
|---|---|
| Total: | 1.6 |
| Professional: | 1.2 |
| Other: | 0.4 |

**Project Description:**

Objectives: The voltage clamp technique allows measurements of ionic current flows across nerve membrane as a function of voltage and time. These parameters have been combined into a set of empirical equations capable of expressing nerve membrane activity, but the physical meaning of the equations is unknown. It is hoped that the use of various chemicals known to effect nervous activity may contribute to understanding the physical mechanisms of the membrane. Irrespective of the chemical properties of the substances, this may permit dissection of the lumped parameters of the equations as well as a correlation of known chemical properties with the observed effects.

Methods Employed: The voltage clamp technique used in these experiments were described previously. For the internal perfusion experiments, modifications to the Tasaki suction perfusion technique were made to allow the use of the voltage clamp technique. This required inserting the coaxial electrode, which consists of an axial current

electrode and a potential measuring probe, into the suction or outflow cannula without clogging the perfusion flow, a task much more difficult for the Woods Hole axon than the Chile axon, because of the smaller size of the Woods Hole axons.

To make this task less difficult, another modification was made which consisted of using two separate electrodes instead of the single coaxial electrode. The axial current carrying electrode was attached to the infusion cannula and a smaller potential measuring electrode was inserted into the outflow cannula.

Major Findings: It has been shown previously that the ammonium ion can pass through both the fast, normally sodium channels, and the slow, normally potassium channels of the squid giant axon. Further experimental work shows that the early rise times and the late approaches to steady state follow the same curves for normal and ammonium substituted axons. This would mean that whatever the processes are that are responsible for the voltage-dependent permeability changes, they are not altered by changing the cation being transported.

Several other ions of the quaternary ammonium type were studied as sodium substitutes (or "sodium-like" ions) completely replacing sodium. Tetraethyl ammonium ion (TEA) produced a small action potential and small inward currents under voltage clamp. Tetramethyl ammonium ion (TMA) produced no action potential and no inward currents. (The recovery from TMA was much faster and better than the recovery from TEA.) TMA is a good substitute for choline as an ion to totally replace sodium (in current separation experiments of the Hodgkin-Huxley type). Tetrodotoxin (TTX), a substance known to block the fast channel (inward sodium current) was also used with TEA and completely blocked the inward current, produced by TEA. With TMA as with choline the outward currents (presumably potassium) through the fast-channel were more pronounced. (TMA in normal artificial sea water does not block inward currents.) TMA hyperpolarized the axon as does choline whereas TEA depolarizes the axon. The quanidinium ion produced an action potential and inward currents. TTX completely blocked quanidinium inward currents. The internal perfusate for all of these experiments was 0.5 M potassium flouride.

Experiments with heavy water ($D_2O$) replacing $H_2O$ in the external media or the internal media were continued. Ficoll, a high molecular weight polymer was

added to produce a more viscous solution to test the hypothesis that the effects produced by the $D_2O$ were not due to the fact that the $D_2O$ is more viscous than $H_2O$. The viscous Ficoll solution did not produce the effects seen by $D_2O$.

Scientific Significance: The results of experiments with the various ion substitutes and with heavy water provide much more evidence that the fast sodium channels and the slow potassium channels are separate and distinct processes. The actions of blocking agents, tetrodotoxin and tetraethyl ammonium tend to favor spatially separated processes. The deuterium appears to affect the membrane directly. It increases $\tau_m$ of the Hodgkin-Huxley process by about 50% which explains the decrease in the amplitude of the peak inward current and the prolongation of the spike potential. This effect may be explained in terms of hydrogen bonding defects and electrically active protonic conduction.

Proposed Course of Action: It is planned to study more "sodium-substitutes" and "potassium-substitutes" of the quaternary-ammonium ion type and others if they can be found to see what their effect is on the nerve membrane. To increase the understanding of the effects of heavy water, experiments are planned with variations in $D_2O$ concentrations, ionic strength, temperature and pH.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-62 LB/MA 936
1. Biophysics
2. Mathematical Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Ionic Permeabilities of Nerve Membranes: Theoretical Investigations.

Previous Serial Number: Same

Principal Investigator: FitzHugh, R.

Other Investigators: Cole, K. S., Lecar, H., Nossal, R., and Shaw, J.

Cooperating Unit: Division of Computer Research and Technology, NIH
Medical Arts and Photography Branch, DRS

Man Years:

| | |
|---|---|
| Total: | 2.3 |
| Professional: | 2.3 |
| Other: | 0.0 |

Project Description:

Objectives: To investigate the bases, consequences, and and extensions of ionic permeability concepts by mathematical analysis and computation.

Methods Employed: Mathematical analyses, with particular emphasis on nonlinear differential equations and optimization theory, are being used in conjunction with the available computing facilities.

Major Findings: The motion picture "Impulse Propagation in a Nerve Fiber" has been completed and prints have been released to the National Medical Audiovisual Center for distribution on loan.

The modeling of voluntary muscular activity has been continued. A muscle described theoretically by the equations of A. V. Hill and connected to various mechanical constraints is assumed to receive a motor input control which minimizes energy expenditure throughout a certain

shortening distance. For a constant or spring force constraint, the optimal control varies continuously with the strength of the constant force. For an inertial force constraint, the control is bang-bang, i.e., the optimal control switches discontinuously from zero to full on and back again. For a damped force constraint, there is no optimal control; as the control function approaches zero, the energy expenditure decreases and the contraction takes more and more time. However, if the control function is exactly zero, it does not permit any contraction at all.

A study has been made of fluctuations in axon thresholds expected for various physical noise sources (done in collaboration with Dr. R. Nossal of DCRT). The object was to obtain a general understanding of how noise operates upon a triggerable device, such as a nerve. Although there have been a number of stochastic models of neural fluctuations in the past, none of them have been sufficiently detailed to make quantitative estimates of the actual sources of noise. In order to see how thermal voltage and conductance fluctuations are changed into threshold and latency variability, the effects of the addition of random terms to the Hodgkin-Huxley equation for a node of Ranview were studied. It was shown that most of the observed phenomena could be obtained from the V,m reduced approximation first introduced by FitzHugh. A number of classical observations can now be understood in terms of the theoretical models.

In order to find the oscillatory nature of the current-space clamped Hodgkin-Huxley axon the two complex and two real eigenvalues of time for the equations were computed for currents from 0.1x to 5.0x rheobase and for temperatures from 6.3°C to 30°C. The variation of frequency, with temperature agreed surprisingly well at rheobase with the only squid membrane data available from Dr. Guttman of Brooklyn College. However most of the frequency measurements were made after a just threshold spike. These agreed well with the just subthreshold frequencies when both were recorded. Computations of the time course at rheobase $\pm$ 1/2% again showed the Hodgkin-Huxley equations to be good representations of the surviving axon. The measurements on oscillation decrement were unsatisfactory but further experiments should give an additional comparison with the computations.

Hodgkin and Huxley separated the clamp current into the sodium and potassium components by changes of external sodium. These effects were illustrated by CEIR computations of the clamp currents as the sodium potential and sodium conductance coefficients were changed according to the Nernst and independence relations over the ranges of -34 mV to +76 mV for clamp potential and 3% to 100% for $[Na]_e$.

For economy the print out was made at approximately logarithmic time intervals. The results have been shown in Membranes, Ions and Impulses.

In lieu of a complete analysis of voltage clamp data into the Hodgkin-Huxley parameters it has become a wide spread practice to rely upon the behavior of the steady state and the peak current maxima as indices of the potassium and sodium characteristics of an axon. The steady state curve is readily obtained by a slow ramp of potential rather than as asymptotes of a series of steps. Preliminary calculations suggest that a modified exponential rise of potential may give a reality and a useful approximation to the peak current curve.

Many 35-millimeter photographs of oscilloscope traces, for example, (of membrane currents during voltage-clamp experiments) are produced in this laboratory. Obtaining numerical values of the recorded currents and correlating the information from various frames on the film is very time-consuming. A device has been built which can be used in conjunction with the LINC computer to perform those tasks with little human assistance. A LINC program capable of moving film and of reading and storing identifying information and graphical data from each frame is nearly completed.

Assistance was given to Dr. L. Goldman of the University of Maryland Medical School in the preparation of a paper describing computations of impulse excitation and propagation in a myelinated nerve fiber, using the membrane equations of Frankenhaeuser and Huxley. The numerical methods previously developed at the National Bureau of Standards for the Laboratory of Biophysics were used by Mr. J. S. Albus of the Goddard Space Flight Center in writing the computer program for this work. A detailed discussion with Dr. Goldman of techniques of dimensional scaling in nerve fibers contributed to the interpretation of the computations in this paper.

Scientific Significance: The hypothesis of optimality in modeling voluntary muscular activity provides a potentially useful theoretical approach which does not require a detailed knowledge (still lacking) of the functioning of the CNS in muscular coordination. The hypothesis is made plausible by the concept of evolutionary selection of CNS functioning which tends to favor survival.

Fluctuation phenomena in nerve have been studied experimentally for some time. Theoretical studies of noise in nerves are useful both to test the applicability of various physical models of membrane conductance and also to understand the limitations which noise sets on neurons as information transmitting devices.

Proposed Course of Project: The solution of the equations for optimal muscular control will be sought for a variety of mechanical constraints acting on a single muscle, a pair of antagonists, or more complicated musculoskeletal systems. It is hoped that the results may provide at least qualitative predictions for comparison with experiment and the eventual production of a coherent theory of coordinated motion.

LINC programs to be completed will obtain data from films, process the data and report results in the form of tables and graphs, and store the data for subsequent analysis.

Honors and Awards: None

Publications:

FitzHugh, R.: Impulse propagation in a nerve fiber, using computer animation. Property of the Laboratory of Biophysics, NINDB,IR,NIH. May be obtained from: Acquisition, Retention and Distribution Center, National Medical Audiovisual Center, Atlanta, Georgia 30333, Oct. 1967.

Serial No. NDB(I)-62 LB/ME 938
1. Biophysics
2. Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Alternating Current Bridge Measurements of Squid Axon Membrane Dielectric Properties.

Previous Serial Number: Same

Principal Investigator: Taylor, R. E

Other Investigators: None

Cooperating Unit: None

Man Years

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: The need for more and detailed measurements of membrane dielectric properties remains, particularly in the low frequency range and the effects of temperature and possible drug and ion effects.

Methods Employed: The presently available methods for space and voltage clamping the squid giant axon are required as well as the developing techniques for internal replacement and perfusion and the use of accurate bridge networks.

Major Findings: None. This project is being held in abeyance until the resources are available to pursue it further.

Scientific Significance: Detailed information on the impedance of the membrane, particularly the voltage and temperature dependence relations, imposes constraints on any molecular model which is proposed. One example is that the membrane does not have the same dielectric properties as the simple bilipid layers which have been worked on.

Proposed Course of Project: Further experiments are planned and will be done when the possibility presents itself. The techniques are available but the squid are to be had only at Marine Biological Stations. Transportation of squid appears to be feasible but the development of the necessary facilities are beyond the budgetary and manpower resources of the Laboratory.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-65 LB/ME 1240
1. Biophysics
2. Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Molecular Biophysics - Physical Properties of Membranes and Simple Membrane-like Systems.

Previous Serial Number: Same

Principal Investigators: Ehrenstein, G., Lecar, H. and Stillman, I.

Other Investigators: Nossal, R.

Cooperating Unit: National Bureau of Standards
Division of Computer Research and Technology, NIH

Man Years

| | |
|---|---|
| Total: | 1.4 |
| Professional: | 1.4 |
| Other: | 0.0 |

Project Description:

Objectives: To study physical properties and molecular structure of well-defined synthetic systems related to natural membranes. To examine related molecular properties of excitable membranes with newer physical techniques.

Methods Employed: Wide line nuclear magnetic resonance spectra are obtained to determine phase changes in myelin (bovine, human, rat and guinea pig), and related synthetic structures, induced thermally and by changes in hydration state. Nuclear magnetic resonance line shapes at low temperatures are studied to determine the state of molecular ordering.

Impedance and noise fluctuation measurements are made on a 10-20 micron diameter patch of squid axon membrane using a new type of cannulation apparatus. Techniques are being developed to make similar measurements on other biological membranes, such as that of

the barnacle muscle, which may be more suitable for noise studies.

Artificial membranes are prepared from beef brain extracts and other material largely according to the methods of Mueller and Rudin. Special cells and mounts are constructed to reduce vibration and noise voltage fluctuations are measured during current flow.

Sulfonated and carboxylated polyanthracenes with respectable cationic-exchange capacities are synthesized. These organic semiconductor materials are examined in specially constructed cells to determine the pKa and monovalent cationic selectivity changes induced by controlled modification of charge density or electric field strength at the ion-exchange sites.

Major Findings: The nuclear magnetic resonance studies of myelin have revealed the existence of thermally induced phase changes in the physiological temperature range. Previously observed structural changes (by X-ray diffraction) have now been shown to be reversible transformations of the hydrocarbon moiety and not protein denaturations. Measurements of line widths at low temperatures provide evidence for tight complexing of phospholipids with cholesterol.

A simple system has been fabricated for making lipid bilayers of resistance about $10^7$ ohm $cm^2$. EIM, the activating factor introduced by Rudin and Mueller, has been prepared by standard bacteriological techniques.

Preliminary measurements were made of impedance and noise voltage fluctuations of a 10-20 micron diameter patch of squid axon membrane. The delineation of this patch must be done from inside the axon because of the thickness of the external Schwann cell layer.

Scientific Significance: Membranes are not only responsible for electrical activity and for the transfer of materials into and out of cells but are implicated in macromolecular synthesis, enzyme activity, the release and effects of hormones, immunological responses and probably in the initiation of antibody production. The importance of increasing our knowledge of the structure and properties of these membranes and simpler membrane like systems is great indeed. Nuclear magnetic resonance studies of appropriate material such as myelin, outer retinal rod preparations and, hopefully, pure nerve membrane fractions

provides detailed information about the state of the hydrocarbon chain region. NMR line width changes are sensitive indicators of mesomorphic phase transitions, which have often been suggested as part of the mechanism responsible for the voltage and hormone dependent permeability changes of cell membranes. Ordered paracrystalline structures have been invoked to explain the remarkable stability of myelin and the breakdown of this stability in certain diseases has been attributed to a weakening of the Van der Waals binding of the hydrocarbon chains. Certain important features of the structures involved may be probed with the low temperature nuclear magnetic resonance studies.

Noise and fluctuation measurements provide a means of estimating the density of conducting channels or sites in an excitable membrane, and hence of finding the resistance of a single ionic channel. The idea of sparsely populated conducting channels imbedded in an insulating membrane has great heuristic value in explaining the nerve blocking effects of minute amounts of drugs such as tetrodotoxin and also explaining many of the ionic permeability phenomena which can be induced in inert phospholipid films by certain polypeptides. The study of the kinetics of conductance changes by means of measuring voltage fluctuations provides an important alternative means of exploring the conducting channel notions.

A detailed study of cationic selectivity as a function of charge density is possible with the use of the organic semiconductor-ion-exchange material discussed above.

Proposed Course of Project: A number of proposals are being considered involving the use of the electron paramagnetic resonance apparatus, all of which require careful temperature control and the attachments for this purpose have been ordered but not yet received. The first of these will be investigations of the mechanism of charge transfer in organic semiconductors of the type which have been used in our laboratory. It is also planned to look at rhodopsin as soon as it is feasible.

Further nuclear magnetic resonance studies of myelin and possibly nerve membrane extracts are planned. If the nerve membrane material becomes available (from the retinal nerves of the Chilean squid) titration of ion exchange groups and measurements of the exchange of deuterium for hydrogen ions will be attempted.

Detailed studies of the properties of the activated lipid bilayers made according to the procedures of Rudin and Mueller are in progress. We are particularly interested in the time course of resistance changes following sudden potential changes and in the effects of varying concentrations of activating factors applied to these bilayers.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1967 through June 30, 1968
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological Diseases and Blindness

Dr. Roscoe O. Brady, Acting Chief

The Laboratory of Neurochemistry is presently subdivided into five sections. The research accomplishments of the respective units during the past year are summarized in this report.

SECTION ON AMINO ACIDS AND ELECTROLYTES: Experimental work in this section was largely curtailed during the past year due to the assignment of the principal investigator as the Acting Associate Director for the NINDB Extramural Programs. A series of experiments dealing with the effect of ouabain on the metabolism of cat cerebral cortex slices was successfully completed. These investigations revealed that the presence of $10^{-5}$M ouabain in the incubation medium caused a 300% increase in mitochondrial $Ca^{++}$ along with an increase in mitochondrial respiration. These observations extend our knowledge of factors which regulate the metabolism of electrolytes in nerve cells. An understanding of these processes in normal brain tissue should provide insight into alterations which occur in pathological states such as epilepsy.

SECTION ON DEVELOPMENTAL NEUROCHEMISTRY: Studies on the mechanism of cerebral edema have been pursued by investigating the relationship between chloride ion concentration and cortical swelling. Studies with slices of cat cerebral cortex revealed that there was a linear relationship between cortical swelling and the concentration of $Cl^-$ in the incubation medium. When isethionate ions ($HO\text{-}CH_2\text{-}CH_2\text{-}SO_3^-$) were substituted for high chloride levels used to induce swelling of the slices, this swelling was reduced by 80%. Initial pharmacological studies with isethionate indicate minimal, if any, toxic effects. After comprehensive pharmacological investigations have been completed, experiments will be undertaken to determine if the administration of isethionate exerts a beneficial effect on cerebral edema.

SECTION ON ENZYME CHEMISTRY: Studies have been extended on the mechanism of action of the $Na^+$-$K^+$ activated ATPase in neural tissues. This enzyme is generally regarded as the principal catalyst involved in maintaining ionic gradients across nerve membranes. Especially relevant data were obtained during the past year on the mechanism of the inhibitory effect of cardiac glycosides on this enzyme. An irreversible complex is formed between cardiac glycosides and the enzyme rendering the enzyme inactive. The formation of this complex is retarded by the presence of $K^+$ ions and accelerated by $Na^+$, $Mg^{++}$, nucleotides and phosphate. When the enzyme is complexed with cardiac glycosides, it can be phosphorylated with inorganic phosphate, a reaction

which does not occur with the native enzyme. These observations provide further indication that the $Na^+$-$K^+$ ATPase may exist in several conformational states. These findings are consistent with the concept that change in the conformation of this membrane-bound enzyme is primarily responsible for cation transport catalyzed by this enzyme. The active site of this enzyme is now being studied in detail in order to determine what structural features are required for catalytic activity and confer substrate specificity.

Experiments have been initiated on ATPases found in red and white muscle tissue. The results obtained in these studies indicate that the lower specific activity of the red muscle ATPase is caused by the presence of an associated modifier material which is complexed or has reacted with red myosin ATPase. It is conceivable that this phenomenon represents a particular form of allosteric inhibition. Clarification of the nature of this complexing substance is of great potential significance for an understanding of metabolic regulatory mechanisms.

SECTION ON LIPID CHEMISTRY: The data obtained in studies carried out in this laboratory which revealed the nature of the metabolic defect in Fabry's disease have now been published. Additional cases were analyzed in the past year and the findings were completely consistent with the previous evidence of the absence of the enzyme, ceramide trihexosidase in these patients. Male hemizygous patients with the full syndrome of Fabry's disease usually die of renal failure in the 3rd or 4th decade of life. Knowledge of the metabolic defect in this disease suggested that amelioration of this condition might be obtained by kidney transplantation. This procedure was recently performed on a patient with Fabry's disease by Dr. U. C. Dubach at the Medical University Clinics in Basel, Switzerland. The patient withstood the surgery satisfactorily and has been in good condition since the operation. It will be of great importance to follow the clinical course of this patient because, if his condition is improved by the transplantation of an organ from a healthy donor, such a procedure may be useful for the treatment of other hereditary lipid storage diseases as well.

Significant progress has been made in the past year in our studies on Tay-Sachs disease. The metabolism of the ganglioside which accumulates in the neurons of these patients has now been demonstrated for the first time in vitro. This development permits the undertaking of experiments which deal with the specific enzymatic reactions involved in the catabolism of this ganglioside. It is hoped that extension of these studies will provide insight into the nature of the metabolic defect in Tay-Sachs disease.

Other studies have indicated that very high concentrations of sphingolipid hydrolases are present in subcellular particles called lysosomes. This finding suggests that the lipid storage diseases in which we have succeeded in identifying the metabolic lesion, namely Gaucher's disease, Niemann-Pick disease and Fabry's disease may fall into the general category of lysosomal diseases.

Studies on the catabolism of sphingolipids in human leukocytes have been successfully extended in several directions. We have succeeded in developing a new and facile diagnostic test for detecting patients with metachromatic leukodystrophy. Secondly, we have demonstrated that there is a marked increase in activity of certain sphingolipid hydrolases in leukemic leukocytes. This finding is to be contrasted with the deficiency of these enzymes in leukocytes obtained from patients with Gaucher's disease and Niemann-Pick disease. The findings in the leukemia studies serve as a starting point for additional experimentation dealing with sphingolipid metabolism in human neoplasms and in cells grown in tissue culture which have been transformed by viruses. Other studies with human leukocyte preparations have demonstrated that these cells catalyze the synthesis of complex lipids as well as their catabolism. These observations were extended to investigations of sphingolipid metabolism in leukocytes obtained from patients with Chediak-Higashi syndrome. The sphingolipid content of leukocytes from these patients is reduced to 50% of the values seen in normal human leukocytes. There was an increase both in the rate of synthesis and catabolism of sphingolipids in leukocytes obtained from patients with this syndrome. The significance of these observations is unclear at this time although it is conceivable that these metabolic disturbances may be related in some way to the eventual occurrence of lymphomas in many of these patients.

Other studies are currently underway in this section on the enzymatic reactions which occur during myelination. A soluble enzyme system has been prepared from young rat brain tissue which catalyzes the biosynthesis of phosphatidic acid, the key intermediate in phospholipid metabolism. Secondly, the effect of puromycin has been examined on the biosynthesis of gangliosides. It was found that the administration of puromycin at levels which inhibited protein synthesis also profoundly depressed ganglioside formation. This effect seems to be rather specific for this class of lipids since the synthesis of less complex lipid materials was unaffected by the injection of puromycin. These observations suggest that the biosynthesis of gangliosides is somehow dependent on the formation of new protein molecules. A concerted set of reactions involving the formation of both of these materials may be required for the biosynthesis of cell membranes.

The Section has developed a high degree of competence in the chemical synthesis of complex lipids labeled with radioactive isotopes of carbon and hydrogen. Recently labeled ceramide trihexoside was synthesized in this laboratory which permitted the discovery of the enzymatic defect in Fabry's disease. In addition, a procedure was developed for the chemical synthesis of radioactive sphingosine, the basic component of all of the sphingolipids. Radioactive ceramide lactoside has lately been made available. It is the key compound for our studies on sphingolipid metabolism in neoplastic tissues.

SECTION ON PHYSIOLOGY AND METABOLISM: A particularly significant series of experiments was carried out in the past year which led to the isolation of a new type of lipid from cell membranes. These materials have recently been shown to be long chain fatty acyl derivatives of pantetheine. Compounds of this nature appear to be confined exclusively to the plasma membrane of cells. They appear to function as an important class of intermediates since they exhibit very rapid metabolic turnover. In fact, they may be the transmembrane carrier molecules for long chain fatty acids. The discovery of this novel class of biologically active materials is truly a major contribution in this area of investigation.

Other studies in this Section have demonstrated that the amino acid methionine plays a key role in lipoprotein synthesis. It has long been assumed that the main contribution of methionine was that of a donor of methyl groups for phospholipid formation. It appears from the present observations that it is also an essential component of the protein moiety of lipoproteins and thus can function in several ways in regulating lipid metabolism.

These investigations were extended using materials from various biological sources in which certain specializations of the function and structure of lipoproteins have appeared phylogenetically. Free fatty acids are transported in the blood stream of the dog fish in association with lipoproteins rather than with albumin as in most other species. Also the red ibis has developed a particular carrier protein in its serum to transport the chromogen which is ultimately deposited in the feathers. Information derived from studies in these special situations should provide a better understanding of the processes involved in the assembly of lipoprotein molecules in serum and during the formation of biological membranes such as the myelin sheath.

Finally, the Section is involved in studies on the comparative biology of neurohumoral substances. Again advantage is taken of special conditions which exist in certain sub-species of sharks. Experiments have shown that catecholamines are synthesized in the interrenal body in these fish which is akin to the adrenal cortex in other species of animals. These data suggest that the adrenal cortex as well as the medulla may play a role in the elaboration of catecholamines.

CRITIQUE: The overall goal of the Laboratory of Neurochemistry is to provide fundamental biochemical data dealing with the structure and metabolism of the nervous system. It appears that this goal has been accomplished in an exemplary fashion during the past year. Significant contributions have been made regarding biochemical events dealing with the normal and abnormal metabolism of neural tissues. A new diagnostic test has been developed for the detection of patients with metachromatic leukodystrophy. The discovery

of the metabolic defect in Fabry's disease has permitted the initiation of a specific therapeutic attempt for the treatment of this disease. Other data have revealed a potential therapeutic agent for the treatment of cerebral edema. Additional investigations carried out in this laboratory during the past year have contributed towards our understanding the pathological events which occur in epilepsy and Tay-Sachs disease. It has been a rewarding and productive year.

Serial No. NDB(1)-61 LNC/AAC 810
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Metabolism of Free and Protein-Bound Amino Acids in Neural Tissues.

Previous Serial Number: Same.

Principal Investigator: Dr. D. B. Tower.

Other Investigators: None.

Cooperating Units: None.

Man Years:

| | |
|---|---|
| Total: | 0 |
| Professional: | 0 |
| Others: | 0 |

Project Description:

Objectives: To investigate the metabolic interrelationships (and factors affecting them) of amino acids, especially of the glutamate and aspartate group, in the free and protein-bound pools of neural tissues, with emphasis on cerebral cortex studied in vitro.

Methods: The time honored in vitro tissue slice incubation method utilizing characterized incubation media with subsequent tissue and media protein and amino acid analytical techniques were employed.

Major Findings: [Work on this project was suspended during this fiscal year while the principal investigator was on temporary duty detail to the NINDB Extramural Programs as Acting Associate Director.]

Significance: This project bears directly upon the fundamental problem of delineating some of the interrelationships between cerebral structure and metabolism, and between metabolism and functional activity, so that an eventual integration of these facets of cellular neurochemistry can be realized.

Proposed Course: To study compositional characteristics of proteins isolated from microsomal membranes induced in liver by phenobarbital and in cerebellum by Dilantin.

Publications:

1. Tower, D.B.: Enzymatic determination of glutamine and asparagine. In: Hirs, C.H.W. (Ed.): Enzyme Structure, Vol XI of Methods in Enzymology, New York, Academic Press, 1967, pp. 76-93.

2. Knizley, H., Jr.: The enzymatic synthesis of N-acetyl-L-aspartic acid by a water-insoluble preparation of a cat brain acetone powder. J. Biol. Chem. 242: 4619-4622 Oct. 1967 .

Serial No. NDB(1)-61 LNC/AAC 811
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electrolytes and Energy Metabolism in Cerebral Cortex in vitro.

Previous Serial Number: Same.

Principal Investigator: Dr. D. B. Tower.

Other Investigators: None.

Cooperating Units: Dr. W. L. Stahl and Mr. Carl Lauter, Section on Physiology and Metabolism, LNC.

Man Years:

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Others: | 0 |

Project Description:

Objectives: To study the in vitro metabolism of electrolytes and of energy-producing cycles and compounds thereof in incubated slices of cerebral cortex.

Methods: The established in vitro method of incubation of subcellular fractions with and without inhibitor solutes was used.

Major Findings: Slices of adult cat cerebral cortex were incubated in usual bicarbonate-saline-glucose-media containing 1.25mM $Ca^{++}$ and 27mM $K^{+}$ with or without added $10^{-5}$M ouabain. After 1 hr. the control and ouabain-treated sets of slices were each pooled, homogenized in 0.32M sucrose, and the mitochondria isolated by method I of Stahl et al. (J. Cell Biol. 19: 293, 1963). Aliquots of the two homogenates and of the two mitochondrial preparations were analyzed for total protein content and for $Ca^{++}$ and $Mg^{+}$ content. No differences in $Mg^{++}$ contents were found. $Ca^{++}$ specific activities in mμM/mg. protein were as follows: homogenates - controls 25.1; ouabain-treated 35.2; mitochondria - controls 13.35; ouabain-treated 43.6. Thus, the hypothesis previously proposed (see 1967 report) is further supported by the 3-fold enrichment of $Ca^{++}$ in mitochondria from the ouabain-treated slices. To recapitulate, we propose that one important action of ouabain on cerebral cortex is the

mobilization of tissue $Ca^{++}$, its increase in tissue and its sequestration by mitochondria, the last aspect being associated with stimulation of mitochondrial respiration.

Significance: Energy-yielding metabolism is the basic factor underlying neuronal function and activity, and electrolyte metabolism (which clearly depends upon it) provides a fundamental link between cellular chemistry and the functional activity of impulse conduction. The understanding of factors involved is essential for the elucidation of both normal functioning of neural tissues as well as deranged functions of hyperactivity states.

Proposed Course: To pursue investigations along lines indicated by previous results.

Publications:

1. Tower, D.B.: Distribution of cerebral fluids and electrolytes in vivo. In: Klatzo, I. and Seitelberger, F. (Eds.): Brain Edema, New York, Springer-Verlag, 1967, pp. 303-332.

2. Tower, D.B.: Inorganic constituents of neural tissues. In: Lajtha, A. (Ed.): Handbook of Neurochemistry, Vol. I, New York, Plenum Press, in press .

3. Tower, D.B.: Ouabain and the distribution of calcium and magnesium in cerebral tissues in vitro. Exper. Brain Research, in press.

Serial No. NDB(1)-6g LNC/DNC 1549
1. Neurochemistry
2. Developmental Neurochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Fluid Dynamics in Cerebral Tissues.

Previous Serial Number: New Project

Principal Investigator: Dr. Robert S. Bourke

Other Investigators: Mr. Oscar Young

Cooperating Units: Biomedical Engineering and Instrumentation Branch

Man Years:

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 2.0 |
| Other: | 0 |

Project Description:

Objectives: To study mechanisms of cerebral edema formation and its control.

Methods: The classical *in vitro* tissue slice incubation method utilizing characterized incubation media and indicator solutes were used throughout.

Major Findings: (1) A number of experiments previously reported have shown that (a) cortical edema formation *in vitro* is related to the concentration of $K^+$ in the incubation medium: a linear relationship obtains between tissue swelling and media $K^+$ concentration over the range 27mM K to 125mM K; and (b) the $K^+$ effect on swelling is dependent on the presence of $Cl^-$ ion in the medium. (Bourke, R. and Tower, D., J. Neurochem. 13: 1071, 1966). In elucidating further the relationship between $K^+$ and $Cl^-$ on swelling of cerebral cortex, the following have been found:

(1) There is a linear relationship between $Cl^-$ concentration in incubation media swelling of cerebral cortex over a range 8mM Cl-132mM Cl with $K^+$ concentration maintained at $\sim$ 54nM/L, and isosmolarity maintained by replacing chloride with isethionate. In addition, slices incubated for 1 hr in the presence of 54nM K and 132mM Cl in a saline-bicarbonate glucose medium ($\sim$300 mOsm) and then transferred to a medium of similar composition and tonicity but with chloride replaced by isethionate lost $\sim$80% of fluid of swelling.

(2) When chloride kinetics were investigated by tracer studies ($Cl^{36}$) in vitro under the above conditions, it was found that at a constant external [$K^+$] the rate of entry of chloride into cerebral cortex after diffusion into the extracellular space followed saturation kinetics with Vmax = 7.7 μM/gm min and Km = 245. The kinetic data so described differ significantly from a curve describing simple diffusion ($P = <.02$). Moreover, a clear dependence on [$K^+$] in incubation media was found. When external chloride was held constant at 6.8 mM/L and [$K^+$] varied over a range of 27 to 100 mM/L, the apparent rate of chloride entry followed saturation kinetics (Vmax = 0.191 μeq/gm/min and Km = 30.3. Again upon investigation of the rate of flux of $Cl^-$ under equilibrium conditions, it was found that chloride influx was not statistically different from efflux and that the extracellular space derived by extrapolation was within the mean (±S.D.) determined for influx, a value essentially identical to that previously determined for chloride space in vivo. (Bourke, Greenberg and Tower, Am. J. Physiol. 208: 682, 1965).

(3) Having thus a clear approximation of the extracellular compartment and having thus described the kinetics of fluid and electrolyte loss (in vitro), it was found that the composition of the fluid of swelling was essentially an isotonic expansion in which the predominant ions were $K^+$ and $Cl^-$. Moreover, it was apparent that the added swelling was membrane found and could be reduced isosmotically by(1) Lowering external $K^+$ (Bourke and Tower: J. Neurochem. 13:1071, 1966) or by replacing external chloride with isethionate. Preliminary in vitro and in vivo toxicity studies indicate minimal, if any, injurious effects resulting from replacement of chloride by isethionate.

Significance: Altered fluid dynamics are concomitants of most of the important clinical problems, whether these be hyperactivity, trauma, vascular or tumor, so that an understanding of factors which determine fluid distribution and a delineation of the sites of distribution are essential to rational therapy. These aspects are of particular importance in the developing brain where there are significant changes in fluid distribution associated with growth and maturation and where there can be frequent superimposition of states of edema, hydrocephalus, and the like.

Proposed Course: To pursue investigators along the lines indicated by present studies, e.g. investigation of the possibility of replacing body chloride by isethionate as a method of combating traumatic cerebral edema (cortical).

Publications:

None.

Serial No. NDB(1)-61 LNC/EC 813
1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Enzymological Aspects of Neural Function.

Previous Serial Number: Same.

Principal Investigator: Dr. R. W. Albers.

Other Investigators: Drs. G. J. Siegel, and F. J. Samaha; and Mr. G.J. Koval.

Cooperating Units: Dr. L. Guth, Section on Exp. Neurology, LNNS, NINDB.

Man Years:

| | |
|---|---|
| Total: | 3.5 |
| Professional: | 2.5 |
| Other: | 1.0 |

Project Description:

Objectives: To assess the functional roles and interrelations of enzyme systems which characterize neural tissue.

Methods: Isotope studies; classical techniques of enzyme purification and characterization; cytological fractionations.

Major Findings: (I) Studies on the mechanism of the $Na^+$-$K^+$ -ATPase have produced considerable insight into the action of cardiac glycosides (CS). Although the effect of these agents on the failing heart has been attributed to their "positive isotropic" action on heart muscle it has been recognized for some years that the most potent and specific action of CS at the molecular level is their inhibition of $Na^+$-$K^+$-ATPase. We have studied the interaction of CS with enzyme preparations from both Electrophorus electric organ and cat brain. Cardioactive steroids react with the enzyme to form a stoichiometric complex which is irreversible under physiological conditions. The rate of formation of the drug-enzyme complex is markedly dependent upon the composition of the incubation medium. Thus $K^+$, which is known to "antagonize" the action of digitalis drugs in vivo, retards the rate of

formation of the complex. Positive effectors are $Mg^{++}$, $Na^{+}$, nucleotides and ortho-phosphate.

The chemical reactivity of the enzyme active site is altered by the combination of CS: the native enzyme is only phosphorylated by ATP, but after combination with CS, the enzyme will react with ortho-phosphate. Extensive comparison has conclusively demonstrated that the same site on the enzyme is phosphorylated in both cases. The implications of this for active $Na^{+}$-$K^{+}$ transport are discussed in the next section.

The role of $Mg^{++}$ in the $Na^{+}$-$K^{+}$-ATPase reaction is under study. We have previously obtained indications that $Mg^{++}$ interacts with the enzyme at more than one site. Recent experiments have shown that the electric organ enzyme requires less than $10^{-5}$M $Mg^{++}$ for complete phosphorylation while the optimum $Mg^{++}$ concentration for hydrolysis is more than 100 times greater. The site with high affinity evidently functions in substrate binding while the low affinity site regulates the configuration of the enzyme.

The chemical characterization of the active site of electric organ $Na^{+}$-$K^{+}$-ATPase appear feasible as a result of recent experiments and technical developments. Methods have been developed for purifying and analyzing millimicromolar quantities of the active site peptide.

(II) The collaborative studies on the nerve muscle relationship have focused on the characteristic differences between the myosin ATPases derived from red and white muscles. Preliminary evidence suggests that the characteristically lower specific activity of the "red" or "slow" myosin ATPase is a result of some modifier associated with the red myosin. Certain activating treatments can raise the specific activity of the red myosin enzyme to that of the white enzyme. Further details will be reported by Dr. Guth (LNS/EN).

Significance: The studies on cardiac glycoside inhibition of $Na^{+}$-$K^{+}$ -ATPase are part of our investigation of the mechanism of the enzyme and its relation to the maintenance of $Na^{+}$ and $K^{+}$ gradients across plasma membranes. The fundamental relationships between this process and neural functions are well known as have been discussed in our previous reports.

The results of these studies appear to support the model for active transport that we have proposed on the basis of our earlier work. We interpret these studies as indicating that a major portion of energy content of the enzyme must be referred to the conformational state of the protein. Many properties of the system can be explained in terms of two metastable states which differ in chemical reactivity at the phosphorylation site and in affinities for $Na^{+}$ and $K^{+}$.

The equilibrium between these states is altered by combination with substrates, products and cations. The stability of the CS-enzyme complex may arise from a partial degradation of the tertiary enzyme structure with resultant inactivation.

This description of "drug-receptor" interaction appears to be unique at present. However, it may be profitable to examine other extremely potent drugs and toxins from this aspect--particularly those that interact with membrane components.

Proposed Course: (1) Several additional experiments which have been suggested by our work on the cardiac glycoside mechanism will be pursued.

(2) A major opportunity to establish the chemical nature of the active site of the enzyme, i.e., the amino acid sequence of the active site peptide, is currently being explored and will probably become a major phase of our work in the next year.

(3) Several questions have arisen in our earlier studies about the $Na^+$-$K^+$-ATPase mechanism which can only be answered by studying reaction times in the millisecond range. We have acquired the necessary equipment and intend to explore some of these points.

(4) We expect to initiate studies directed toward the structural and regulating aspects of the active transport system as it is integrated into the membrane.

There are indications that "electrogenic" cation transport may be an important part of the electrical activity of some nerve cells. This implies properties of the enzyme which have not yet been observed.

Appropriate studies will require membrane preparations from these cells which are often available in only minute quantities.

(5) We expect the myosin ATPase project in collaboration with the Section on Experimental Neurology, LNNS, to assume greater importance in our section in the coming year as the biochemical problem becomes better defined.

Publications:

1. Siegel, G.J., and Albers, R.W.: Sodium-potassium-activated adenosine triphosphatase of Electrophorus electric organ. IV. Modification of responses to sodium and potassium by arsenite plus 2,3-dimercapto-propanol. J. Biol. Chem., 242, 4972-4970, Nov. 1967.

2. Fahn, S., Koval, G.J., and Albers, R.W.: Sodium-potassium activated adenosine triphosphatase of Electrophorus electric organ. V. Phosphorylation by adenosine triphosphate-$^{32}$P. J. Biol. Chem. 243, 1993-2002, May 1968.

3. Albers, R.W., Koval, G.J., and Siegel, G.J.: Studies on the interaction of ouabain and other cardioactive steroids with sodium-potassium-activated adenosine triphosphatase. Mol. Pharmacology in press, May 1968 .

Serial No. NDB(1)-61 LNC/LC 815
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Metabolism of Complex Lipids of Nervous Tissue. Studies on the Nature of the Metabolic Defects in Gaucher's Disease, Niemann-Pick Disease, Fabry's Disease and Metachromatic Leukodystrophy.

Previous Serial Number: Same.

Principal Investigator: Dr. R. O. Brady.

Other Investigators: Drs. A. E. Gal, N. Weinreb, A. K. Percy, and Mr. R. M. Bradley

Cooperating Units: Dr. David Shapiro, Dept. of Organic Chemistry, Weizmann Institute of Science, Rehovoth, Israel.

Man Years:

| | |
|---|---|
| Total: | 1.9 |
| Professional: | 1.4 |
| Other: | 0.5 |

Project Description:

Objectives: (1) To elucidate the biosynthetic pathways for the formation of long chain fatty acids, cerebrosides, gangliosides, and sphingomyelin; (2) to study the control mechanisms which regulate these processes; and (3) to study the metabolic fate of sphingolipids in normal and lipodystrophic disease states.

Methods: Glucocerebroside and galactocerebroside labeled with $^{14}C$ in either the hexose or fatty acid portion of the molecule have been synthesized. $^{14}C$-labeled sphingomyelin and gluco-and galactopsychosine have been similarly prepared. Ceramide-trihexoside and ceramide tetrahexoside (globoside) uniformly labeled with radioactive hydrogen-$^{3}H$ have been prepared. The metabolism of these labeled materials has been investigated in vivo and in vitro.

Major Findings: The enzymes which catalyze the hydrolysis of glucocerebroside and sphingomyelin were demonstrated by experiments in this laboratory to be markedly diminished in tissues of patients with Gaucher's disease and Niemann-Pick disease respectively. These findings were extended to enzymatic investigations on washed leukocyte preparations from small samples of venous blood. These latter experiments led to the development of novel and reliable tests for the diagnosis of Gaucher's disease and Niemann-Pick disease.

With this information as a background, a facile and accurate procedure was recently developed for the diagnosis of metachromatic leukodystrophy (MLD) (1). This test depends on the differential determination of the activity of the enzyme arylsulfatase A in carefully prepared leukocytes. These cells also exhibit arylsulfatase B activity, but only the former enzyme is affected in MLD. The activity of arylsulfatase A in leukocyte preparations from 6 patients with MLD was less than one-tenth of that found in the control series of leukocyte preparations which included samples from patients with several other sphingolipidoses. A statistical analysis of the data showed that the difference in the mean activity of this enzyme in the two populations was highly significant ($p = <.0005$).

The data obtained in our study on the nature of the metabolic defect in Fabry's disease has now been published (2). Additional cases have been analyzed during the current year, and the findings on these patients were completely consistent with the reported deficiency of ceramide trihexosidase in Fabry's disease. A diagnostic test using intestinal biopsy specimens has been developed for the detection of this disease and for discovering females who carry the genetic lesion responsible for Fabry's disease.

Critical studies were undertaken during the past year on the subcellular distribution of various sphingolipid hydrolases (3). The results obtained in these experiments indicated that all of the enzymes examined exhibited their highest specific activity in subcellular particles identified as lysosomes.

Significance: The demonstration of the nature of the metabolic defects in Gaucher's disease, Niemann-Pick disease and Fabry's disease and the development of facile diagnostic tests for detecting these sphingolipidoses represent major contributions in this field of medical investigation. The U. S. Government has filed application for patent rights on the diagnostic tests for Gaucher's disease and Niemann-Pick disease. In addition, the test devised in this laboratory for the diagnosis of

metachromatic leukodystrophy is another important contribution in this area. The relative ease and reliability of this procedure is a vast improvement over previously existing diagnostic methods such as the quantitative estimation of urinary sulfatide excretion or the histochemical examination of biopsy specimens of the sural nerve.

The demonstration of the nature of the metabolic defect in Fabry's disease has led to the first attempt to treat this condition by transplantation of a kidney from a healthy donor to a patient with Fabry's disease.

The finding of high concentrations of sphingolipid hydrolases in lysosomes provides new insight into the growing body of knowledge regarding lysosomal diseases. This information may be of special significance in a consideration of therapeutic procedures for the amelioration of sphingolipidoses.

Proposed course: At this point we wish to examine in even greater detail the explicit nature of the enzymatic changes in sphingolipidoses. In order to accomplish this objective, we are initially attempting to completely purify the enzyme glucocerebrosidase which is involved in Gaucher's disease. We need to know if there is an abnormality in the amino acid sequence of this enzyme in patients with Gaucher's disease. We have already observed significant differences in the substrate affinity and heat-stability of the enzyme obtained from these patients compared with enzyme preparations from normal human tissue sources. These findings appear to indicate that structural changes have occurred in the enzyme molecule in patients with Fabry's disease. Knowledge of the exact nature of these aberrations should be helpful for devising procedures to treat these diseases.

Publications:

1. Percy, A.K., and Brady, R.O.: Metachromatic leukodystrophy: Diagnosis with venous blood samples. Science, in press.

2. Brady, R.O., Gal, A.E., Bradley, R.M., Martensson, E., Warshaw, A.L., and Laster, L.: Enzymatic defect in Fabry's disease: ceramide-trihexosidase deficiency. New Eng. J. Med. 276: 1163-1167, May 1967.

3. Weinreb, N.J., Brady, R.O., and Tappel, A.L.: The lysosomal localization of sphingolipid hydrolases. Biochim. Biophys. Acta, 159: 141-146, May 1968.

4. Kanfer, J.N., Brady, R.O.: Rat liver sphingomyelinase. In Colowick, S.P. and Kaplan, N.O. (Eds.): Methods in Enzymology, New York, New York, Academic Press, Inc. In press.

5. Brady, R.O.: Enzymatic abnormalities in diseases of sphingolipid metabolism. Clinical Chemistry, 13: 565-577, July 1967.

6. Brady, R.O.: Disorders of cerebrosides and disorders of sphingomyelin. In Shy, G.M., Goldenson, E.S., and Appel, S.M. (Eds.): The Cellular and Molecular Basis of Neurologic Diseases. Philadelphia, Pa., Lea and Febiger, in press.

7. Brady, R.O.: Disorders of sphingolipid metabolism. In Ehrenpres, S. and Solnitzky, O. (Eds.): Neurosciences Research Vol. II. New York, New York, Academic Press, in press.

8. Brady, R.O.: Enzymatic defects in the sphingolipidoses. In Dodansky, O., and Stewart, C.O., (Eds.): Advances in Clinical Chemistry, Vol II, New York, New York, Academic Press, Inc., in press.

9. Brady, R.O.: The sphingolipidystrophies. In Bondy, P.K., (Ed.): Duncan's Diseases of Metabolism, VI Edition, Philadelphia, Pa. W. B. Saunders, Co., in press.

10. Brady, R.O.: Lipidoses. In Lajtha, A., (Ed.): Handbook of Neurochemistry, Vol. III, New York, New York, Plenum Publishing Corp., in press.

11. Brady, R.O.: The metabolism of sphingolipids. In Bolis, L., and Pethica, B.A., (Eds.): Membrane Models and the Formation of Biological Membranes. Amsterdam, The Netherlands, North-Holland Publishing Co., in press.

Serial No. NDB(1)-61 LNC/LC 816
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Structural and Metabolic Studies of Gangliosides in Normal Humans and Patients with Tay-Sachs Disease.

Previous Serial Number: Same.

Principal Investigator: Dr. J. N. Kanfer.

Other Investigators: Drs. R. O. Brady, A. E. Gal, E. Kolodny, and Miss J. Quirk.

Cooperating Units: None.

Man Years:

| | |
|---|---|
| Total: | 2.1 |
| Professional: | 1.2 |
| Other: | 0.9 |

Project Description:

Objectives: To investigate the structure and metabolism of gangliosides.

Methods: Tay-Sachs ganglioside was isolated and purified from pathological specimens. A portion was subjected to tritiation by the Wilzbach technique. Unlabeled carrier was added and the mixture purified by conventional chromatographic techniques. The final product was found to cochromatograph with authentic standard in four different solvent systems. Aliquots of this radioactive ganglioside were injected intracerebrally into 10 day old rats. The animals were returned to their mothers and sacrificed three days later. The brains were removed and the lipids isolated.

Major Findings: (1) In vivo - The only radioactive ganglioside recovered was found to be unchanged Tay-Sachs ganglioside. In addition, labeled ceramide, ceramide monohexoside, and ceramide dihexoside were isolated, indicating metabolism of Tay-Sachs ganglioside in brain in vivo.

(2) In vitro - The metabolism of labelled Tay-Sachs ganglioside has recently been demonstrated in a particulate fraction from rat intestine. Preliminary evidence has indicated an extensive degradation of the substrate. The individual reaction products are currently being isolated and identified. In addition, the various enzymes responsible for the catabolism of Tay-Sachs ganglioside are being examined.

Significance: Since the only recovered material in studies in vivo with $^{3}$H-labeled Tay-Sachs ganglioside was the starting material, this would argue against the suggestion by various workers that the Tay-Sachs ganglioside is a precursor of the other brain gangliosides. In addition, since several other sphingoglycolipids were isolated, it would appear that the brain has a complement of necessary enzymes which catalyze the hydrolysis of the various carbohydrate moieties of Tay-Sachs ganglioside. It is hoped that these observations may eventually aid in elucidation of the metabolic disturbance in Tay-Sachs disease. The recent studies in vitro should now afford sufficient information to allow the undertaking of a program to elucidate the enzymatic defect in Tay-Sachs disease.

Proposed Course: To pursue studies on the metabolism of Tay-Sachs-$^{3}$H ganglioside in vitro.

Publications:

1. Kanfer, J.N., and Brady, R.O.: Studies on the biosynthesis of gangliosides. In Volk, B. W. and Aronson, S. (Eds.) Inborn Errors of Sphingolipid Metabolism. New York, N. Y., Pergamon Press, 1966, pp. 187-192.

Serial No. NDB(1)-61 LNC/LC 817
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Immunochemical Studies in Multiple Sclerosis.

Previous Serial Number: Same.

Principal Investigator: Dr. R. O. Brady.

Other Investigators: Dr. N. Weinreb.

Cooperating Units: Dr. Mitsuo Yokoyma, Kuakini Research Foundation, Honolulu, Hawaii; Dr. T. Borsos, Biology Branch, National Cancer Institute; Dr. P. Nelson, Laboratory of Neurophysiology, NINDB.

Man Years:

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0 |

Project Description:

Objectives: To determine whether auto-immune phenomena participate in the pathogenesis of neurological diseases.

Methods: Immunological examinations were made on sera from patients with multiple sclerosis and animals with experimental allergic or viral encephalomyelitis for the presence of antibodies to gangliosides, asialogangliosides, myelin sheath lipids, etc. Animals were immunized by several procedures with various ganglioside preparations.

Major Findings: Data obtained in the course of this study indicate that anti-ganglioside antibodies have a marked inhibitory effect on syntaptic impulse transmission. This inhibition is reversible and is restricted to an effect on post-synaptic neurons. Various ganglioside preparations exhibit distinctly different antigenic potency and the resultant neurophysiological effects vary accordingly.

Significance: The use of immunological probe outlined in this report may provide information regarding the chemistry of the receptor site on

the post-synaptic neuron. Such knowledge would represent a fundamental contribution towards the elucidation of the processes involved in synaptic impulse transmission.

Proposed course: Additional anti-ganglioside antibody preparations will be obtained and examined for their effects on synaptic transmission. The effect of various relatively simple inhibitory materials will be examined in order to try to obtain insight into the chemical nature of the receptor site on the post-synaptic nerve membrane.

Publications:

None.

Serial No. NDB(1)-66 LNC/LC 1308
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Sphingolipid Metabolism in Tissue Culture Cells and in Leukocytes.

Previous Serial Number: Same.

Principal Investigator: Dr. J. N. Kanfer.

Other Investigators: None.

Cooperating Units: Dr. R. A. Yankee, Medicine Branch, NCI.

Man Years:

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: To conduct a program designed to study the metabolism of complex sphingolipids in homogeneous mammalian cell populations. The cell lines available at present are principally from peripheral organs and blood. The ultimate goal will be to work with neural cells.

Methods: Examination of the metabolism of various radioactive sphingolipids and sphingolipid precursors in certain cell lines, as specified below.

Major Findings: Intact human leukocytes were prepared by published techniques, incubated with either glucose-$^{14}$C or galactose-$^{14}$C for 3 hrs, and the sphingolipids were extracted and separated by silicic acid column chromatography and preparative thin-layer chromatography. The carbohydrate moieties were liberated by acid hydrolysis and characterized both by paper chromatography and carrier dilution.

The principal sphingoglycolipids found to be labeled were a glucosylceramide and mixture of digalactosyl- and lactosyl-ceramides. It was demonstrated that glucose-$^{14}$C and galactose-$^{14}$C were both efficiently converted to the glucose portion of the glucose cerebroside. In a similar manner both glucose-$^{14}$C and galactose-$^{14}$C were observed to be

converted to the galactose of the ceramide dihexosides. In these more complex sphingoglycolipids only a slight amount of radioactivity was detected in glucose. In addition, a ganglioside-like material was also formed in these incubations. Thus far the nature of the radioactivity in this material is uncertain.

Significance: The findings in cells from tissue culture and in leukocytes of biosynthetic and degradative enzymes for the various sphingoglycolipids provides a ready source and convenient model with which to study sphingoglycolipid metabolism and to diagnose disorders thereof. There are many technological difficulties associated with such studies in brain that may be overcome by work on pure cell lines and subsequent application to brain samples. In addition leukocytes provide an interesting preparation with respect to phagocytosis and the presumably associated elaboration of cellular membranes with which sphingolipids are associated.

Proposed Course: Project is completed and will be discontinued.

Publications:

1. Kampine, J.P., Martensson, E., Yankee, R.A., Kanfer, J.N.: Sphingolipid metabolism in leukocytes I. Lipids 3 151-156, March 1968.

Serial No. NDB(1)-66 LNC/LC 1309
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on the Metabolism of Sphingolipids in Tumor Tissues.

Previous Serial Number: Same.

Principal Investigators: Drs. R. O. Brady and A. E. Gal.

Other Investigators: Mr. R. M. Bradley.

Cooperating Units: Dr. Chloe Tal, Laboratory of Experimental Neurology, Hadassah University Hospital, Jerusalem; Dr. M. Dolan, Dept. of Pathology, National Naval Medical Center; Drs. P. Mora, General Laboratories and Clinics, NCI, and G. Todaro, Etiology Branch, NCI.

Man Years:

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 0.5 |
| Other: | 0.5 |

Project Description:

Objectives: To determine the metabolic pathways of sphingolipids in neoplastic tissues.

Methods: Various complex sphingolipids are synthesized which are labeled with $^{14}C$. The metabolic fate of these materials is studied in vivo and in vitro in normal and neoplastic tissue preparations.

Major Findings: The activity of enzymes which catalyze the hydrolysis of sphingolipids was determined in leukocyte preparations from normal individuals and patients with acute and chronic myelogenous leukemia. A marked increase in the activity of glucocerebroside-cleaving enzyme was observed in the leukocyte preparations from the patients with both forms of myelogenous leukemia. Sphingomyelin-cleaving enzyme activity was also elevated in leukocytes from patients with myelogenous leukemia.

We have begun investigations on the metabolism of sphingolipids on tissue culture using primary cell cultures and neoplastic cell lines obtained by transforming cells with SV-40 and polyoma virus. Our observations on sphingolipid metabolism and these preparations are consistent with the changes observed in leukemic leukocytes. We have initiated similar studies with neoplastic tissues from human sources.

Significance: These observations indicate some fundamental differences in sphingolipid metabolism in rapidly growing and neoplastic tissues when compared with normal tissues. Of particular interest is the possible relevance of these observations to the lack of contact inhibition exhibited by neoplastic cells. The elucidation of the nature of these metabolic alterations may shed considerable insight into the processes which occur in oncogenesis.

Proposed Course: The enzymes which catalyze the synthesis and catabolism of other, more complex sphingolipids will be investigated in tissue culture preparations obtained from normal and neoplastic tissue sources.

Publications:

1. Kampine, J.P., Brady, R.O., Yankee, R.A., Kanfer, J.N., Shapiro, D., and Gal, A.E.: Sphingolipid metabolism in leukemic leukocytes. Cancer Research 27: 1312-1315, July 1967.

Serial No. NDB(1)-67 LNC/LC 1457
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Chemical Synthesis of Radioactive Sphingolipids.

Previous Serial Number: None.

Principal Investigator: Dr. A. E. Gal.

Other Investigators: Mr. F. Fash.

Cooperating Units: None.

Man Years:

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 1.0 |
| Other: | 1.0 |

Project Description:

Objectives: To prepare sphingolipids labeled with radioactive isotopes of carbon and hydrogen for metabolic studies in hereditary lipid storage diseases and neoplastic conditions.

Methods: Chemical procedures have been devised for the synthesis in good yield of DL-erythro-sphingosine and DL-threo-sphingosine specifically labeled with radioactive hydrogen-$^{3}H$ in positions 4 and 5 of the molecule. The labeled sphingosines were quantitatively separated from acetylenic impurities and the physical constants of the pure products were determined. D-erythro-sphingosine was prepared by resolution of the DL racemate.

Procedures have been devised for the non-specific labeling of sphingolipids. This technique has the advantage that highly complex molecules can be made radioactive and their metabolism studied even though the specific chemical synthesis of the compounds is not feasible. For example, galactosylgalactosylglucosyl-ceramide (ceramide trihexoside) was labeled by treating this material with a large excess of tritium.

The products obtained by this procedure contained many radioimpurities. Complex purification procedures were devised and used in order to obtain the desired sphingolipid in a highly purified form. This material was suitable for metabolic studies.

In order to expedite the analytical methods involved in the identification of the structure of various glycolipids. New thin-layer chromatography procedures were devised for the qualitative and quantitative determination of the various monosaccharides residue of the sphingolipids. New methods were also developed for the quantitative determination of radioactivity in various carbohydrate constituents of the sphingolipids.

Major Findings: The preparation of labeled ceramide trihexoside permitted the successful undertaking of studies which culminated in the demonstration of the nature of the metabolic defect in Fabry's disease in this laboratory. (See Project Report LNC/LC #815). An extension of these studies have resulted in the development of diagnostic procedures for the detection of patients with Fabry's disease and heterozygous females which are carriers of this genetically linked enzymatic deficiency.

Significance: Enzymatic studies with labeled sphingolipids have now come into prominence for elucidating metabolic defects in hereditary lipid storage diseases. Suitably labeled sphingolipids are also of great value in the diagnosis of patients with these conditions and many laboratories and clinics throughout the world have sought our help in providing them with such diagnostic studies. The tests are facile and reliable and generally cannot be performed wihout the use of labeled materials. It is anticipated that the extension of these procedures will ultimately lead to the pre-natal diagnosis of patients with lipid storage diseases by performing enzyme studies on cells obtained by aminocentesis.

Proposed Course: Using the techniques of specific and non-specific labelling, other sphingolipids such as Cytolipin H (lactosylceramide) will be labeled. Further work will be done on the Tay-Sachs ganglioside. This molecule cannot yet be synthesized chemically. Efforts are concentrated on a basically new approach, which is the exchange of an (inactive) functional group by a $^{14}C$-labeled identical group. It is hoped that approximately labeled material will be obtained in this fashion which will facilitate studies on the nature of the biochemical defect in Tay-Sachs disease.

Publications:

1. Gal, A.E.: Synthesis of DL-erythro and threo-sphingosine-4,5-$^{3}$H. J. of Labelled Compounds 3: 112-119, April 1967.

2. Gal, A.E.: An improved method for the detection and radioassay of monosaccharides. J. of Chromatography 34: 266-268, April 1968.

3. Gal, A.E.: Separation and identification of monosaccharides from biological material by thin-layer chromatography. Anal. Biochem., in press .

Additional publications listed in project reports LNC/LC 815, LNC/LC 1309, and LNC/LC 1458.

Serial No. NDB(1)-67 LNC/LC 1458
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effect of Puromycin in vivo on the Incorporation of Labeled Precursors into Sphingoglycolipids in Young Rat Brains.

Previous Serial Number: None.

Principal Investigator: Dr. J. N. Kanfer.

Other Investigators: Dr. A. E. Gal, Miss R. Richards, Mr. R. M. Bradley.

Cooperating Units: None.

Man Years:

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.1 |
| Other: | 0.1 |

Project Description:

Objectives: To determine the effect of puromycin on the incorporation of labeled precursors into brain sphingoglycolipids.

Methods: Radioactive sugars (glucose-$^{14}C$, galactose-$^{14}C$, galactosamine-$^{14}C$) and the sphingosine base (DL-erythro sphingosine-$^{3}H$) were injected intracerebrally into young rats. Experimental animals received intracerebral injections of puromycin while the control animals received saline. Four hours later the animals were sacrificed, the brains removed and the radioactivity in the various sphingoglycolipid was measured.

Major Findings: Puromycin was found to cause an approximate 70% depression in the incorporation of the radioactive sugars and sphingosine-$^{3}H$ into the brain gangliosides. Concommitantly there was only a 20-25% decrease in the labeling of the other sphingoglycolipids from the labeled sugar precursors. However, with sphingosine-$^{3}H$ which has been previously demonstrated to be converted only to ceramide and sphingomyelin, puromycin was demonstrated to result in a 150-200% increase in the conversion to brain sphingomyelin.

Significance: Puromycin has been demonstrated by other workers to inhibit cerebral protein synthesis and to interfere with the maze learning abilities of rats. Attempts have therefore been made to correlate protein synthesis and the memory process. The studies summarized in this report indicates that aside from the inhibition of protein synthesis puromycin can drastically alter the ability of the brain to synthesize sphingolipids. Such an alteration of lipid metabolism might result in impaired learning ability in animals.

Proposed Course: Project is completed and will be discontinued.

Publications:

1. Kanfer, J. N., and Richards, R.: Effects of puromycin on the incorporation of labeled sugars into gangliosides in vivo. J. Neurochem. 14: 513-518, May 1967.

2. Kanfer, J.N., Bradley, R.M., and Gal, A.E.: Effect of puromycin on the incorporation of erythro DL $^{3}H$ sphingosine into sphingolipids in vivo. J. Neurochem. 14: 1095-1098, Sept. 1967.

Serial No. NDB(1)-67 LNC/LC 1459
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Conversion of L-α-Glycerol Phosphate-$^{14}$C into Phosphatidic Acid by a Soluble System from Rat Brain.

Previous Serial Number: None.

Principal Investigator: Dr. J. N. Kanfer.

Other Investigators: None.

Cooperating Units: None.

Man Years:

| | |
|---|---|
| Total: | 0 |
| Professional: | 0 |
| Other: | 0 |

Project Description:

Objectives: To partially purify and establish certain properties of a soluble system from young rat brain which catalyzes the acylation of L-α-glycerol phosphate to yield phosphatidic acid.

Methods: L-α-glycerol-$^{14}$C phosphate was synthesized by the enzymatic phosphorylation of glycerol-$^{14}$C. This material was then employed as substrate in order to purify an enzyme system which catalyzes the transfer of palmitic acid from palmityl CoA to form phosphatidic acid. Conventional methods of enzyme purification were employed.

Major Findings: By the use of a Lourdes mechanical electric homogenizer a measurable amount of activity could be detected in the 100,000 x g supernatant prepared from rat brain. The activity could be precipitated with ammonium sulfate at 20-40% of saturation. In order to obtain optimal activity the addition of boiled, dialyzed 100,000 x g supernatant was required. The products were isolated by column chromatography and the principal compound obtained was demonstrated to be phosphatidic acid. The system required ATP; palmitoyl CoA or palmitic acid + CoA; L-α-glycerol phosphate and enzyme. Some of the characteristics of the systems were elucidated.

Significance: This is the first demonstration of a system which forms phosphatidic acid from L-α-glycerol phosphate in the soluble portion of the cell. Previous studies of this reaction were limited to microsomal and mitochondrial systems and therefore this is the first attempt at any purification of the enzyme responsible for this reaction. The nature of the heat stable non-dialyzable material in the 100,000 x g supernatant is not clear at present. It is conceivable that this may be analagous to the acyl carrier protein of bacterial system, which appears to be involved in acyl transfer.

Proposed Course: Project is completed and will be discontinued.

Publications:

1. Martensson, E., and Kanfer, J.N.: The conversion of L-glycerol-$^{14}$C-3-phosphate into phosphatidic acid by a solubilized preparation from rat brain. J. Biol. Chem. 243, 497-501, Feb. 1968.

Serial No. NDB(1)68 LNC/LC 1550
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Myelination in the Central Nervous System.

Previous Serial Number: None, new project.

Principal Investigators: Drs. R. O. Brady and R. A. Snyder

Other Investigators: None.

Cooperating Units: Dr. M. Brightman and J. Cammermeyer, LNNS, NINDB
Dr. I. Tasaki, LNP, NIMH; Drs. A. Rabson and
R. Malmgren, PA, NCI.

Man Years:

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 1.0 |
| Other: | 0 |

Project Description:

Objectives: To investigate biochemical events related to the formation and maintenance of the myelin sheath of nerves. In particular, to establish the exact enzymological reactions which occur during the initiation of myelin deposition, to determine how the integrity of the myelin sheath is maintained, and to obtain an understanding of the biochemical pathology which occurs in demyelinating and dysmyelinating lesions in the nervous system.

Methods: Procedures will be developed for obtaining individual cell types from mammalian cerebral cortices. The chemistry and metabolism of these specific cell populations will be investigated using analytical techniques specially developed in this laboratory. Particular attention will be devoted to the biochemical events which occur in the various cells at the time of myelination.

Major Findings: We have succeeded in devising a procedure which permits, for the first time, the preparation of individual, viable homogenous cell types from brain tissue. These cells will now be analyzed for their individual structural and metabolic characteristics.

Significance: The development of a satisfactory method for obtaining individual cell types from mammalian brain tissue is a major breakthrough and has been the object of much experimental effort in a number of laboratories in this country and abroad. It permits for the first time the undertaking of critical experiments on the chemistry of the individual species of cells in brain. In particular, this development should provide a logical approach for the examination of the role and contribution of the various types of cells to the formation and maintenance of the myelin sheath. Such information is of particular importance for investigating biochemical abnormalities in pathological conditions such as multiple sclerosis and hereditary demyelinating diseases.

Proposed Course: The homogeniety of the various cell preparations will be established by rigid characterization of the respective fractions. When stringent criteria have been satisfied in this regard, various analytical and biochemical (metabolic) studies will be undertaken with the individual cell species. Particular attention will be devoted to the biochemical events associated with myelination. Experimentation will be extended to investigations of experimental myelinopathies in lower animals and in genetically mutated mice. It is anticipated that the information obtained in the course of these studies will point the way to relevant studies in human diseases.

Publications:

None.

Serial No. NDB(1)-68 LNC/LC 1551
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Alteration of Sphingolipid Content and Metabolism in Leukocytes from Patients with the Chediak-Higashi Syndrome.

Previous Serial Number: None.

Principal Investigator: Dr. J. N. Kanfer.

Other Investigators: None.

Cooperating Units: Drs. R. S. Blume and S. M. Wolff, Laboratory of Clinical Investigation, NIAID; and Dr. R. A. Yankee, Medicine Branch, NCI.

Man Years:

| | |
|---|---|
| Total: | 0.4 |
| Professional: | 0.4 |
| Other: | 0 |

Project Description:

Objectives: To investigate sphingolipid content and metabolism in leukocytes from patients with Chediak-Higashi Syndrome.

Methods: Peripheral leukocytes were obtained from patients with Chediak-Higashi disease. These were analyzed for:

1. Total sphingolipid content by techniques developed in this laboratory.
2. Ability to incorporate radioactive glucose-$^{14}C$ and $^{32}P$ into sphingolipids.
3. The levels of glucosylceramide and sphingomyelin hydrolase activity by procedures originated in this laboratory.

Major Findings: The sphingoglycolipid and sphingophospholipid content from the patient material was found to be decreased to 50% that present in normal leukocytes. There is a marked increase in the ability of the leukocytes from patients to incorporate radioactive precursors into sphingolipid. In addition, studies have revealed that there is an increased ability of leukocytes from these patients to cleave both glucosylceramide and sphingomyelin.

Significance: The hallmark of the Chediak-Higashi syndrome is the presence of enlarged granules which are presumed to be lysosomes in all organs of the afflicted individual. The findings of decreased sphingolipid content coupled with an increased metabolism of the sphingolipids may be an indication of an increased leukocyte turnover. Somewhat similar observations were obtained in myelogenous leukemia leukocytes (LNC/LC 1309). These metabolic alterations may be a reflection of a fundamental biochemical change in both types of leukocytes since, the pathologic course in many patients with Chediak-Higashi syndrome eventually results in the development of lymphomas.

Proposed Course: Project is completed.

Publications:

1. Kanfer, J.N., Richards, R., Kampine, J.D., Handmaker, S., and Yankee, R.A.: Alteration of sphingolipid content in leukocytes from patients with Chediak-Higashi syndrome. Life Sciences 6: 2661-2664, Dec. 1967.

Serial No. NDB(1)-62 LNC/PM 941
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Bioelectrogenesis.

Previous Serial Number: Same.

Principal Investigator: Dr. E. Trams.

Other Investigator: Dr. W. Stahl.

Cooperating Units: None.

Man Years:

| | |
|---|---|
| Total: | 0 |
| Professional: | 0 |
| Other: | 0 |

Project Description: Discontinued and in part incorporated into LNC/PM 1481.

Serial No. NDB(1)-65 LNC/PM 1242
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Mechanism of Lipoprotein Synthesis.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: Mr. Carl J. Lauter.

Cooperating Units: Dr. E. A. Brown, Lab. Chem. Pharmacology, NHI.

Man Years:

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 0.2 |
| Other: | 0.8 |

Project Description:

Objectives: To investigate the mechanism of lipoprotein synthesis as a potential model for the assembly of lipid-protein interphases in membranes.

Methods: Lipoprotein synthesis is studied in the rat by using isotopic precursors for the protein and lipid moieties of the complex lipoprotein particle (micelle). The plasma lipoproteins are obtained by flotation in the ultracentrifuge at different densities. The incorporation of free fatty acids (FFA) into the lipoprotein lipids is measured after chromatographic separation of the major lipid classes. The delipidized lipoproteins are subjected to acid hydrolysis and their amino acid composition is determined. Incorporation rates of various amino acids into the protein is measured in fasting animals and in animals which are synthesizing lipoproteins actively following a diet load. A pilot study was conducted on the lipoprotein composition of the spiny dog fish, and similarly on the lipoproteins and the carotenoid carrier proteins of the red ibis.

Major Findings: Using an automated Amino Acid Analyzer system, the incorporation of 20 $C^{14}$-labeled amino acids into rat plasma lipoproteins was measured. The rats were exsanguinated at various times (up to 6 hrs) following the intravenous injection of an isotopic amino acid mixture. After fractionation of the plasma lipoproteins into their major density classes, the lipoproteins were delipidated and the residual proteins subjected to acid hydrolysis. The liberated amino acids were separated on the Auto-analyser, their radioactivity measured and the specific activity of each amino acid calculated. Our previous contentions that methionine played a significant role in lipoprotein biosynthesis were substantiated by the findings that this amino acid was exceedingly well utilized for lipoprotein synthesis. It had been assumed generally, that methionine serves primarily as a methyl donor (i.e. lecithin synthesis), but our observations indicate that its major role is that of an essential component in protein synthesis per se. Wehther or not methionine serves a function in determining the lipid composition of lipoproteins remains to be established.

A somewhat different role for lipoproteins as lipid-carriers was suggested by observations on the dog fish Squalus acanthias. In this animal, the protein, free fatty acid, triglyceride, cholesterol, and phospholipid content of the plasma lipoproteins was determined. It was found that the chylomicrons and the very low density lipoprotein fractions carried the major portion of the lipids. The free fatty acid content of the lipoprotein fractions was considerably higher than in the comparable mammalian fractions and it is conceivable that this species utilized lipoproteins as the FFA carrier. This is in marked contrast to most mammalian species where the FFA are transported in an albumin-bound form.

Distribution of the phosphatides in the various lipoprotein classes revealed no unusual patterns, and the phospholipids were qualitatively and quantitatively quite similar to those found in rats or man.

During the Alpha Helix Amazon expedition we did have occasion to work on the plasma lipoproteins of the red ibis (eudocimus ruber). This particular bird contains appreciable amounts of carotenoid pigments in the blood plasma. A significant portion of these pigments are utilized by the animal for plumage coloration and the maintenance of this (bright red) color depends upon adequate, exogenous, supplies of carotenoids. By use of thin layer chromatography and spectrometric techniques it was established that the half life of the major carotenoid chromophore (guaraxanthin) was about 100 hours. Fractionation of the ibis plasma proteins revealed that the carotenoid carrier was associated with the

high density lipoproteins. Further separation of this fraction by disc electrophoresis showed that the carotenoid carrier could be separated from the bulk of the high density lipoproteins, and studies on the carotenoid carrier complex are presently underway. Of particular interest have been some observations which were made subsequently on some ibis in the National Zoo, Washington, D. C. In the aviary collection there, we were able, and from one rare hybrid which is thought to be an offspring of a red-white mating. Preliminary data indicated that the white ibis was unable to synthesize the carrier, but failed to incorporate the plumage chromophores. If the permission of the D. C. Zoo can be obtained, attempts will be made to make antibodies to the carotenoid carrier. The purpose would be to make an attempt to suppress "carotenoid-carrier" synthesis or utilization. It is possible, that such experiments may lead to a better understanding (and perhaps control) of faulty human lipid metabolism.

The investigations outlined above not only will yield a better insight into the structure and the metabolism of the plasma lipoproteins, but should also indicate by what mechanisms peptides and lipids aggregate into definite complexes of biological significance. This project has developed, because some of our current thinking on the structure and function of the plasma membrane indicates, that it may provide us with useful models. We have postulated that the membrane is composed of a lipid-bilayer with protein covering certain portions of the bilayer and also interdigitating with the lipid phase in some areas. The concept was that a template mechanism or a coding principle exists which will determine the composition of the lipid phase. It was reasoned that a similar, if not identical, problem exists in the formation of the plasma lipoproteins and probably the myelin sheath of nerves. The study of lipoproteins synthesis appeared to represent a logical approach to this problem and was selected, since a facile experimental approach seemed feasible.

Significance: Lipoprotein-lipid composition can not be related solely to total amino acid composition, but as the resolution of the method improves, more such relationships will become apparent. For instance, on the basis of the amino acid analyses and the isotope incorporation experiments, it might be speculated that methionine could play a significant overall role in the biosynthesis of the low density lipoproteins. Finally, a thorough understanding of lipid transport mechanisms may influence future clinical approaches to disorders of lipid metabolism.

Proposed Course: It is proposed to continue this investigation along the lines described above. Further studies will be made of the in vivo and in vitro incorporation of isotopic precursors. In what fashion the proteins or amino acids serve as determinants of the lipid composition in the lipoproteins will be the subject of further studies.

Publications:

1. Lauter, C.J., Brown, E.A. and Trams, E.G.: Composition of plasma lipoproteins of the spiny dogfish Squalus acanthias. Comp. Biochem. Physiol. 24: 243-247, Dec. 1967.

Serial No. NDB(1)-65 LNC/PM 1243
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Pilot Studies and Consulting Services in Neurochemistry.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: Mr. C. J. Lauter.

Cooperating Units: (see below).

Man Years:

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|---|---|
| Total: | 0.5 |
| Professional: | 0.3 |
| Other: | 0.2 |

Project Description:

Objectives: To provide for various NINDB-IR programs and investigators a focal point for consultation, advice and assistance on neurochemical and biochemical aspects of research problems. To develop new approaches and test new methods; to study non-recurring problems amenable to current biochemical approaches; and as appropriate, to train and/or supervise technical or sub-professional personnel and research associated in such areas.

Methods: As required (see below).

Projects Prosecuted during FY 68:

1. For the Section on Clinical Neuropathology, NINDB, (Dr. E. Rasmussen) facilities were made available and collaboration provided for the use of isotopic tracer and high resolution chromatographic techniques. In particular, some methods were worked out for the study of urea metabolism in the dogfish.

2. For the Section on Cerebral Metabolism, LCS, NIMH (Dr. L. Sokoloff) additional amino acid analyses on protein hydrolyzates were carried out. One of Dr. Sokoloff's technicians was trained in the use and operation of the Phoenix Amino Acid Analyzer.

3. For the Section on Ophthalmology Chemistry, NINDB (Dr. Marc Lewis) analyses of the lipid composition of a rhodopsin preparation were carried out.

4. For the Laboratory of Biophysics, NINDB (Dr. R. E. Taylor) consultations in the preparation and purification of the EIM (excitability inducing molecule) of Rudin and Mueller.

5. For the Section on Amino Acid Metabolism, LNC (Dr. Robert Bourke) methods for the isolation and purification of isethionic acid.

6. For the Section on Chemistry, LM, NHI (Dr. H. Fales) malonylthiophenol was synthesized.

7. For the Adult Psychiatry Branch, NIMH (Dr. R. Colburn) access to the atomic absorption spectrophotometer and the emission flame was provided for analyses of brain-samples for various heavy metals.

8. For the Section on Psychiatry, LCS, NIMH (Dr. Durell) access to the atomic absorption spectrophotometer was provided for analyses of $Li^+$ levels in sera from patients on experimental lithium therapy.

Proposed Course: To be discontinued as a separate project.

Publications:

None.

Serial No. NDB(1)-67 LNC/PM 1480
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Metabolism of Neurohumoral Transmitter Substances in Marine Animals.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: None.

Cooperating Units: Dr. E. A. Brown, Lab. Chem. Pharmacology, NHI.
Dr. D. Rall, LCP, NCI.

Man Years:

| | |
|---|---|
| Total: | 0.15 |
| Professional: | 0.15 |
| Other: | 0 |

Project Description:

Objectives: This study represents an attempt to follow the development of neurohumor transmitter substances and their metabolism in animals lower on the evolutionary scale.

Pelagic organisms have developed not only variants of transmitter substances (such as octopamine) but also a variety of potent blocking agents (neurotoxins) and it is hoped that a better understanding of their structure and metabolism may aid in elucidating the function of neurohumoral transmitters.

Methods: Enzymes which participate in oxidation, reduction, and methylation reactions are studied in vitro in fresh or frozen tissue preparations. Characteristics of the various enzymes are determined by standard techniques and, in a number of tissues, partial purification of certain enzymes is routinely done. Metabolic reactions usually are studied by methods which have been developed for mammalian systems. Frequent use is made of isotopic tracers. The incorporation of labeled precursors or substrates into organs, tissue fractions or

biochemical intermediates is measured in vitro by conventional methods, usually after intravenous administration. In vitro studies, in contrast, are more directed towards the effect of a tissue, cell, or subcellular element on a drug, precursor, or metabolite. The major part of this study was concerned with metabolic reactions in the chondrichthyes, because this class represents a fairly linear evolutionary development from late Middle Devonian times. There have been opportunities, moreover, to make collections of otherwise fairly inaccessible animals (Alpha Helix, Amazon Expedition) and some studies were conducted on fish obtained in the Amazon.

Major Findings: Measurement of methyltransferase activity in the elasmobranch interrenal body indicated that transmethylation reactions in this tissue may have an important function in catecholamine metabolism. Assays for catechol-O-methyltransferase (COMT) and phenyl-N-methyl-transferase (PNMT), by use of S-adenosyl-$C^{14}$-methionine, showed the presence of both enzymes in interrenal body homogenates from various sharks and rays. Both epinephrine and norepinephrine occur in this tissue in substantial amounts. This is rather surprising, considering that the interrenal body in the elasmobranch is the equivalent of the adrenal cortex. This observation led us to conclude that in this species, the interrenal body (i.e. cortex) might be a major source of epinephrine. This hypothesis was further strengthened by studying the in vivo distribution of $H^3$-norepinephrine in nurse sharks. The interrenal body, next to the kidney was the most active organ in concentrating the catechol. Further studies indicated that labeled norepinephrine had been converted to the N-methylated catechol (epinephrine) in significant amounts. Both epinephrine and norepinephrine were metabolized further by the standard reactions involving the O-methyltransferase and the monoamine oxidase systems.

During the Alpha Helix Amazon Expedition in the Fall of 1967 we had occasion to keep a variety of typically Amazonian fish for experimental and observational purposes. The animals referred to were the electric eel (E. electricus), various piranha species, and two species of fresh water elasmobranchs. These fish have to adapt to larger seasonal environmental changes than pelagic species, and it was thought of interest to measure a variety of detoxication reactions which had been reported to be low or absent in fishes. The Azoreductase systems of the livers of the Amazonian fish were more active than those of marine animals, which could mean, that enzymatic levels in detoxication reactions may in part be dependent upon the habitat of a class or species.

Significance: Certain advances, arrests or specializations in the evolutionary process can facilitate the task of the biologist in studying certain complex mechanisms. Some pelagic species, such as the elasmobranchs, have not evolved significantly since Eozoic times. Thus some insight may be gained into the paleobiology of some biochemical processes and their evolution. In particular, this investigation may lead to a better understanding of the role of adrenal cortical steroids in catecholamine metabolism. Conceivably, transmitter substances, such as the catechols, are synthesized under the control of the corticoids. Conversely, the release of transmitter substances, through commands from higher centers, may modulate the subsequent release of corticoids. The juxtaposition of adrenal cortex and medulla in the higher vertebrates may represent an advantageous evolutionary process, and in consequence, migration of enzymes, such as PNMT, into the medullary tissue from cortical tissue, may be the expression of an evolutionary improvement.

Proposed Course: We hope to conduct more extensive studies on the role of the cortical steroids and the interrenal body in chondrichthyes. In essence, very little is known about the transmitter functions of the catechols in this class and it is expected that more light can be shed on this aspect by means of collaborative studies.

If feasible, we shall make an attempt to trace the evolution of catecholamine metabolism through some representative species. In addition we propose to examine the role and metabolism of the catechols in the sympathetic nervous system of the elasmobranch.

Present plans call for a collaborative effort between NINDB and NCI to provide a small biochemistry-pharmacology facility on the grounds of the Mote Marine Laboratory in Sarasota, Fla. This facility (a pilot program) would be jointly operated by LNC, NINDB and LCP, NCI and it is expected that investigators in other institutes will make use of this small laboratory eventually. The purpose of a basic marine biochemistry-pharmacology laboratory would be to give the increasing number of NIH investigators, who make use of marine materials, access to equipment and supplies which will be adequate for most operations.

Publications:

1. Trams, E.G. and Brown, E.A.: Metabolic alterations of catecholamines and other compounds in elasmobranch tissues. Proc. Soc. Exp. Biol. & Med. 125, 253-256, July 1967.

2. Brandenburger Brown, E.A. and Trams, E.G.: Catecholamine metabolism in the shark interrenal body. Proc. 22nd Ann. Meeting Soc. Gen. Physiol. 26, Sept. 1967.

3. Trams, E.G.: Some drug metabolizing enzymes in Teleosts and elasmobranchs from the Amazon Delta. Prog. Rep. Alpha Helix Amazon Expedition, Dec. 1967, D 21-23.

4. Brown, E.A. and Trams, E.G.: Catecholamine metabolism in elasmobranch interrenal body. Comp. Biochem. Physiol. 24, June 1968.

E. G. Trams, Member of the 1967 "Alpha Helix" Amazon Expedition.

Serial No. NDB(1)-67 LNC/PM 1481
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on the Composition and Metabolism of Isolated Cellular Membranes.

Previous Serial Number: None.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: Dr. W. Stahl, Dr. J. Skidmore.

Cooperating Units: None.

Man Years:

| | |
|---|---|
| Total: | 1.65 |
| Professional: | 1.65 |
| Other: | 0 |

Project Description:

Objectives: This project is designed to further our understanding of the composition, structure and function of the cell membrane. The primary objective has been to elucidate the interrelationship of membrane structure to impulse propagation and to the phenomenon of bioelectrogenesis. In the past, however, numerous obstacles have been encountered in attempts to isolate adequate amounts of neuronal membranes, such as giant axon membranes or pure myelin sheath. For this reason, membrane structures of other organs have been examined as methods become available for their preparation. The problem subsequently was approached from two directions: 1) An analytical study was made of the components of isolated cell membranes and 2) certain functions of the membranes were studied in an effort to isolate the principal molecules participating in these processes.

Methods: In recent years, improved methods have become available which allow for the preparation of tissue fractions which represent reasonably pure plasma membranes or have a fairly high proportion of the membranes of various subcellular structures. As specified under the section on Findings, isolation of these membrane fractions was carried

out by the best available techniques and a series of analytical studies was carried out to determine the lipid, protein and carbohydrate composition of these fractions. For these experiments, rats were injected with radioactive precursors and the turnover rates for a number of the component building blocks are estimated in order to establish an index for metabolic activity (or participation in events). As a second approach, the plasma membranes are isolated and synthesis or degradation reactions are followed by using radioactive substrates.

Major Findings: In one series of experiments, plasma membranes were prepared from rat liver homogenates. The membrane preparation was characterized by its composition and the presence of particular enzymes which have been associated with membrane function. An analysis of the amino acid composition revealed no significant patterns which might lead to a better understanding of the function of membrane proteins. The number of individual proteins, however, which can be obtained from the membranes in a soluble form, probably exceeds 25, and a characteristic composition may not be assigned until these proteins are studied as individual membrane elements.

The lipid composition of the membranes was studied in detail and was found to resemble those reported for other biomembranes. Subsequently studies were conducted in which the metabolism of the membrane lipids was investigated in vitro with the aid of isotopic precursors. Palmityl-, stearyl-, oleyl and linoleyl coenzyme A esters were prepared enzymatically from radiocarbon labeled acyl precursors. These substrates were incubated with the membranes and the incorporation of the acyl moiety into various complex lipids was measured. Under these conditions, small amounts of glycerides were formed and the unsaturated acyl derivatives of CoA were better utilized than their saturated analogues. Significant amounts of fatty acid were incorporated into phosphatidic acid, phosphatidylethanolamine and phosphatidylcholine. The characteristics of these reactions led to the proposal that the major phospholipids were formed by reacylation of their lysoderivatives.

Since the biosynthetic pathways leading to the synthesis of the major phospholipids are well understood, attempts were made to study the contribution of these pathways in the isolated membranes to membrane lipid synthesis. It was found that $ATP^{32}$, $^{3}H$-choline, $^{14}C$-β,β-diglyceride and $^{14}C$-α-glycerophosphate were not well, if at all, utilized for phospholipid synthesis.

During these studies it was observed that the incubation of liver plasma membranes with acyl coenzyme A esters yielded a new and unidentified product. The formation of this material seemed to take place only,

if relatively pure membrane preparations were employed. Further studies indicated that this material was liberated by incubation with liver cell membranes, adipose cell ghosts and a membranous fraction prepared from the electric organ of Electrophorus electricus. When erythrocyte ghosts or membranes obtained from intestinal brush border were used, this material was not formed in significant amounts.

Subsequently it was shown, that the unknown lipid in question was S-acyl pantetheine. Although certain acyl pantetheine derivatives had been used experimentally, substituting for coenzyme A, the enzymatic formation of a long chain S-acyl pantetheine had not been reported previously.

A considerable time of this project was allocated for the investigation of the formation of this long chain acyl thioester. Positive identification of S-acylpantetheine was eventually achieved by a combination of mass spectrometry, chemical analyses and by the chemical synthesis of S-palmitylpantetheine.

Significance: Following the finding that long chain acyl coenzyme A esters were converted to S-acyl pantetheine in the presence of some membrane preparations, increased emphasis was placed on certain facets of lipid metabolism in the membrane. In principle, the reaction leading to the formation of S-acylpantetheine is closely related to the $Na^+$ transport of sugars and other metabolites. Presently we are investigating the possibility, that S-acylpantetheines are actually serving as "Acyl Transfer Lipids." Since the mechanisms by which lipids, lipoproteins and especially free fatty acids (FFA) are transported across the cell membrane are not understood, further experimentation on this aspect might lead to significant new insights. If the concept that all transport mechanisms have one or several fundamental reactions in common is correct, then an understanding of one transport phenomenon may lead to a better comprehension of all of them.

Proposed Course: A more detailed study will be made of the mechanism by which S-acylpantetheines are formed. Further, it is planned to appraise the biological significance of this reaction. It is hoped that the answers for the following questions can be provided by a continuation of our present project:

1. What is the exact lipid composition of various biomembranes?
2. How is membrane lipid composition regulated?
3. Does the membrane lipid composition influence or determine its function(s)?

4. How do lipids (free fatty acids, neutral lipids, lipoproteins) pass through the plasma membrane? Diffusion? Active Transport?

Facilitated diffusion? As Protein Complex? As a high energy phosphate- or thio ester? etc.

Eventually, a concept which encompasses the basic elements of a variety of known transport mechanisms may be formulated.

Publications:

1. Trams, E.G.: Thin layer chromatography of hydroxamic acids. J. Lip. Res. 8: 698, Dec. 1967.

2. Stahl, W.E.: $^{14}$C-ADP-ATP exchange activity and the $Na^+$, $K^+$ -stimulated ATPase system. Arch. Biochem. Biophys. 120: 230-231, Nov. 1967.

ANNUAL REPORT
July 1, 1967 through June 30, 1968
Laboratory of Molecular Biology
National Institute of Neurological Diseases and Blindness
Intramural Research

Ernst Freese, Chief

## A. Mutagenic and Inactivating Alterations of DNA

Agents which induce chromosome breaks and large chromosome alterations have been shown to inactivate transforming DNA without inducing point mutations. Conversely, it has been postulated that all agents which induce such "inactivating DNA alterations" will induce chromosome breaks if they are able to penetrate the cellular and nuclear membranes and thus react with the chromosomal DNA. As also shown by the inactivation of viruses or bacteria, DNA synthesis cannot proceed across drastically altered DNA (in which a base has been removed or the backbone has been broken) unless a repair mechanism restores the structure of DNA to its native state. Chemicals containing certain structures are known to produce radicals to inactivate DNA and to induce chromosome breaks. It can therefore be assumed that other chemicals, having the same essential structures, will have the same effects. To verify this contention the DNA inactivating effect of several antidepressant hydrazines is described in the following. Since many of the radical-producing agents react with oxygen, giving rise to OH radicals, the chemical effect of these radicals on DNA and its components has been examined in detail.

### 1. Inactivating DNA Alterations Induced by Antidepressant Hydrazines and Isoniazid.

The hydrazines isocarboxazid, nialamide, and phenelzine are used as drugs against depression, and isoniazid is employed against tuberculosis. The psychotropic effect of the hydrazines apparently results from the inhibition of a monoamine oxidase, whereas isoniazid kills mycobacteria tuberculosis. All these agents have been shown to react with oxygen, giving rise to hydrogen peroxide and intermediate radicals. They also inactivate transforming DNA at concentrations of $10^{-2}$ or $10^{-4}$ M. All these reactions depend on the presence of transition metals and they are inhibited by radical quenchers such as $FeCl_3$, pyrophosphate, or high concentrations of EDTA. The effect of these agents on chromosomes is not known.

### 2. The Chemical Effect of Hydrogen Peroxide on DNA and Oligonucleotides.

In the presence of trace metals, hydrogen peroxide gives rise to OH radicals, which previously have been shown to inactivate transforming DNA rapidly. $H_2O_2$ reacts with DNA in several ways: it opens the 5,6 double bond of pyrimidine bases, produces 7-N-hydroxyadenine which remains attached to DNA, causes the removal of all four bases from DNA, and subsequently results in the breakage of the DNA backbone. The reaction products have been identified by nuclear

magnetic resonance and infrared spectra, by paper chromatography of radioactive products, and by classical chemical tests. By the use of oligodeoxyadenylic acid, it has been proven that the backbone breakage of DNA occurs by β-elimination adjacent to the unstable deoxyribonic acid.

3. Mutagenic Effect of Hydroxylated Adenines.

Since radical-producing agents have a very small mutagenic effect, the mutagenicity of several hydroxylated adenines was examined, using bacteriophage T4. One of these agents, 6-hydroxyaminopurine, was found to be mutagenic.

B. Control Mechanisms and Differentiation

Differentiation in higher organisms depends on many genetic and biochemical mechanisms which are also found in microorganisms, particularly in the process of bacterial sporulation. Since bacteria grow and differentiate faster than higher organisms and mutants can be easily produced, the examination of differentiation is greatly facilitated in these organisms. Typical phenomena observed in all differentiating organisms are the existence of specific genes involved only or predominantly in differentiation, the commitment to continue development once it has been started, the sequential process of development, and the stoppage of DNA synthesis, while RNA and protein continue to turn over. These basic phenomena of differentiation are therefore examined with respect to the sporulation of Bacillus subtilis. Differentiation also involves the intricate control of specific RNA and protein synthesis, which can best be studied by the examination of individual molecular species, e.g. specific enzymes.

1. Control of the Rate of RNA Synthesis.

The overall rate of RNA synthesis depends on the product of the number of new RNA chains started per time interval and the average rate at which these chains grow. Overall RNA synthesis occurs much faster in a rich than in a poor medium, but the rate at which individual RNA chains grow remains the same in both media. In fact, all RNA chains, messenger, ribosomal, and transfer RNA are elongated at the rate of 50 nucleotides per second at 37°C. The growth medium controls, therefore, the frequency with which new RNA chains are initiated. The remarkably slow rate of chain elongation requires that each gene specific for the 23S ribosomal RNA is simultaneously described by many RNA polymerase enzymes. The increased rate of RNA synthesis in rich media must be due to an increased number of RNA polymerase molecules, because the fraction of genes complementary to 23S ribosomal RNA has been measured to be the same in rich and poor media.

2. Amino Acid Control of RNA Synthesis.

It is well-known that all 20 amino acids must be present in order to maintain a normal rate of RNA synthesis. The lack of any amino acid, in a mutant deficient for it, immediately stops RNA synthesis. In Escherichia

coli, "relaxed mutants" have been isolated, which continue their RNA synthesis in the absence of all amino acids. Experiments on plasmolyzed cells, i.e. cells permeable to nucleotide triphosphates, have shown that the amino acid control does not involve RNA polymerase, but rather affects the substrates for RNA synthesis. During amino acid starvation all four nucleotide triphosphates are greatly reduced in amount, indicating that amino acid starvation causes the lack of energy (ATP). An unusual nucleotide has been discovered in thin-layer chromatography of amino acid-starved cells. This nucleotide is destroyed immediately upon addition of the missing amino acid. It is not produced in the relaxed strain. The nucleotide, whose nature is being analyzed, seems to function therefore as a general repressor of RNA synthesis and perhaps of oxidative phosphorylation.

3. Control of Specific Enzymes.

The synthesis of α-galactosidase and thiomethyl-galactoside permease is coordinately induced by three α-galactosides, melibiose, melibiitol, and galactinol. Genetic experiments have shown that both of these enzymes are part of the common operon for which constitutive mutants have been found.

Mutants in another enzyme, dihydrofolate reductase, exhibit an unusual control mechanism. Isolated by their resistance to trimethaprim, they exhibit greatly increased amounts of enzyme. Yet their mutations are not located in an operator gene, but they are part of the structural gene for this enzyme, because the mutant enzymes have altered pH optima or heat stability. Apparently, a mutation within the structural gene can affect the amount of enzyme produced by the cell. The molecular mechanism of this phenomenon will be analyzed.

4. Biochemical Identification and Curing of Mutants Unable to Sporulate.

Sporulation mutants can be divided into two classes, those for which uracil incorporation into RNA can be elevated by any added carbon source and others for which only certain carbon sources give a response. Mutants of the latter type are blocked in different enzymes of the citric acid cycle. Mutations in aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinic dehydrogenase, and fumarase have been identified. Surprisingly, mutants blocked in the latter three enzymes can still grow vegetatively on glucose minimal medium. This shows that the complete citric acid cycle is not absolutely needed for vegetative growth, but it is indispensable for sporulation. Sporulation can also be specifically inhibited in the standard sporulating strain by low concentrations of α-picolinic acid. This compound was found to inhibit specifically aconitase, apparently by chelating ferrous ions that are needed for aconitase activity.

Whereas most sporulation mutants blocked within or outside the citric acid cycle could not be restored to sporulate by the addition of several carbon sources, one mutant could be cured in the presence of acetate, palmitate, or a few other carbon sources. This mutant also produces a very small amount of an antibiotic which is typically produced by B. subtilis during sporulation. The nonresponding mutants, in contrast, do not produce any

antibiotic activity. It is possible that the antibiotic production is necessary for sporulation. The biochemistry of mutants blocked outside the citric acid cycle and the phenomenon of commitment are under investigation.

5. Characterization of Early Reactions in Germination.

Spores of B. subtilis can be initiated to germinate by L-alanine alone. It has now been shown that initiation can also be obtained by the combination of L-asparagine, fructose, glucose, and potassium. Asparagine and fructose undergo some reaction, which subsequently causes the spores to respond to the addition of glucose and potassium much more rapidly than without pretreatment. The results show that several enzymes must be present in spores which allow the metabolic conversion of germination compounds to produce necessary but so far unknown metabolites.

Serial No. NDB(I)-62 LMB/OC 947
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Structure and Alteration of DNA and Chromosomes

Previous Serial Number: SAME

Principal Investigators: Elisabeth Bautz Freese, Ph.D. and Ernst Freese, Ph.D.

Other Investigators: Hans-Jürgan Rhaese, Ph.D.

Cooperating Units: Laboratory of Metabolism, NHI

Man Years:

| | |
|---|---|
| Total: | 3.5 |
| Professional: | 2.2 |
| Other: | 1.3 |

Project Description:

Objectives: 1. A large number of chemical agents induce chromosomal breaks in animals and tissue cultures. In order to determine whether these agents act directly on DNA, their ability to inactivate transforming DNA was investigated. Special emphasis was placed on agents which produce radicals, either directly, by reaction with oxygen, or indirectly after enzymic conversion into radical-producing compounds. 2. Many of the radical-producing agents give rise to OH radicals, whose detailed chemical effect on DNA and its components was investigated. These experiments showed that hydroxylated adenines are produced in the course of the reaction. Consequently, the mutagenic effect of different hydroxylated adenines was measured.

Methods Employed: A special system of transforming DNA has been used to measure both the induction of point mutations and the inactivation of DNA simultaneously. The details of this method have been described previously. The production of hydrogen peroxide was measured by the titanous sulfate method. Oligodeoxyadenylic acid was chemically prepared and purified by column chromatography. The reactions of DNA, oligodeoxyadenylic acid, and nucleotides with hydrogen peroxide were measured spectrophotometrically, by paper chromatography, and by standard chemical methods. The nature of the reaction products was ascertained by nuclear magnetic resonance and infrared spectroscopy. The mutagenic effect of hydroxylated adenines was determined by a spot test technique employing the reversion of phage T4 mutants.

Major Findings: 1. Inactivating DNA alterations induced by antidepressant hydrazines, isoniazid, and other peroxide-producing agents. Hydrazines containing an N-N group, as well as hydroxylamines having a free NOH group, have been shown to produce hydrogen peroxide and thus radicals in the presence of oxygen. This effect is prevented by radical-quenching agents, excess $Fe^{+++}$, EDTA, pyrophosphate, or catalase. In the presence of oxygen, these agents also inactivated transforming DNA, showing the same zero order concentration dependence that had been found earlier for hydroxylamine itself.

In particular, the hydrazines isocarboxazid, nialamide and phenelzine, which are used as antidepressants, and isoniazid, which is employed against tuberculosis, have all been shown to produce hydrogen peroxide and induce inactivating DNA alterations. Agents that produce inactivating DNA alterations in vitro usually induce chromosome breaks in vivo, except when they are inactivated by enzymes in the cell. It is therefore possible that the above pharmaceutical agents induce chromosome breaks. Whether the pharmaceutical doses employed are high enough to significantly increase the frequency of chromosome breaks in humans can be evaluated only by an elaborate statistical analysis.

2. The reaction of hydrogen peroxide with DNA, oligodeoxyadenylic acid, and DNA components. $H_2O_2$ has been shown to react with DNA in several ways: a) It mainly reacts with pyrimidine bases, reducing the UV absorption by opening up the 5,6 double bond of these bases. The UV absorption of purine bases is also destroyed to a small extent, the reaction product being unknown. b) 7-N-hydroxyadenine is produced by hydroxylation of adenine on DNA. The chemical nature of this compound has been established by infrared and nuclear magnetic resonance spectra, by reduction with Raney nickel, and other chemical methods. c) $H_2O_2$ causes the removal of four bases from DNA or from nucleotides by hydroxylation of the 1 position of deoxyribose. By the same reaction the 7-N-hydroxyadenine is removed from DNA. The liberation of the bases subsequently leads to the opening of deoxyribose, giving deoxyribonic acid, which then causes the breakage of the sugar-phosphate backbone. The use of oligodeoxyadenylic acid has shown that breakage involves β-elimination wherever that is possible, whereas cyclization takes place only when β-elimination is impossible.

3. Mutagenic effect of hydroxylated adenines. Several adenines, hydroxylated at different positions, were examined for their point mutagenic effect on bacteriophage T4. The induction of reverse mutations of mutants carrying a base pair transition from AP to GC or vice versa, were measured by a spot test as well as quantitative analysis. One of the agents, 6-hydroxyaminopurine, was found to be mutagenic.

Proposed Course of Project: Analyze the effect of other chromosome-breaking agents on DNA, especially methylating the ethylating agents. Determine whether they break DNA only indirectly by altering first the DNA bases or whether they can cause DNA backbone breakage also directly.

Significance to Bio-medical Research and the Program of the Institute: 60% of neurological diseases are genetically inherited. Some (if not many) of the mutations responsible for these defects may have been induced in the parents by drugs, pesticides, or other chemicals ingested or present in the environment. The knowledge of such chemicals and their reactions with DNA may help to avoid them.

Honors and Awards: None

Publications:

Freese, E. B.: Der Chemismus der Urethan induzierten Mutationen, 25 Jahre nach der Entdeckung. Molec. Gen. Genetics 100: 150-158, Nov. 1967.

Rhaese, H. J., and Freese, E.: Chemical analysis of DNA alterations. I. Base liberation and backbone breakage of DNA and oligodeoxyadenylic acid induced by hydrogen peroxide and hydroxylamine. Biochim. Biophys. Acta 155: 476-490, Feb. 1968.

Rhaese, H. J., Freese, E., and Melzer, M.: Chemical analysis of DNA alterations. II. Alteration and liberation of bases of deoxynucleotides and deoxynucleosides induced by hydrogen peroxide and hydroxylamine. Biochim. Biophys. Acta 155: 491-504, Feb. 1968.

Freese, E. B.: The mutagenic effect of hydroxylaminopurine derivatives on phage T4. Mutation Res. 5: 299-301, March-Apr. 1968.

Freese, E., Sklarow, S., and Freese, E. B.: DNA damage caused by hydroxylamines and antidepressant hydrazines. Mutation Res. In press.

Rhaese, H. J.: Chemical analysis of DNA alterations. III. Isolation and characterization of adenine oxidation products obtained from oligo- and monodeoxyadenylic acids treated with hydroxyl radicals. Biochim. Biophys. Acta. In press.

Serial No. NDB(I)-65 LMB/OC 1208
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Ribonucleic Acid and the Regulation of Cellular Metabolism.

Previous Serial Number: SAME

Principal Investigator: Robert A. Lazzarini, Ph.D.

Other Investigators: Robert M. Winslow, M.D. and Kiyoi Nakata, Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 3.0 |
| Professional: | 2.5 |
| Other: | 0.5 |

Project Description:

Objectives: The nucleic acid content of cells is subject to control by at least several regulatory mechanisms. The synthesis of certain species of messenger RNA is elicited by the presence or absence of specific metabolites in the cell milieu. The accumulation of ribosomal RNA, however, does not respond to the availability of specific nutrients but responds, rather, to the fitness of the medium and the growth rate of the cell. The cellular content of transfer RNA, on the other hand, responds neither to specific nutrients nor to the growth rate but is constant under most conditions of growth. The details of the mechanisms underlying these observations are at present obscure and are the subject of this project. We have concentrated on three aspects of these control mechanisms: 1) Control of RNA synthesis in nongrowing (chloramphenicol-treated) cells. 2) Rate of assembly of RNA chains. 3) Quantitation of genes specific for ribosomal RNA.

Methods Employed: Standard biochemical techniques are employed for the purification of RNA, DNA and enzymes. Analysis of complex mixtures of RNA are performed by chromatography on Sephadex G-100 or methylated albumin or polyacrylamide gel electrophoresis. Messenger RNA is estimated by molecular hybridization with homologous DNA. The rates of synthesis of individual RNA species are determined from the kinetics of incorporation of

radioactive precursors into the species.

Major Findings: 1. Control of RNA synthesis in chloramphenicol-treated cells: Following the addition of chloramphenicol to growing cells, protein synthesis halts immediately but RNA accumulation continues for some time before slowing and finally ceasing. It has been reported by others that this curtailment of RNA synthesis is not co-ordinate and that the synthesis of ribosomal RNA is preferentially inhibited. To explain these results it was suggested that the synthesis of ribosomal RNA but not the synthesis of transfer RNA was regulated by the availability of a protein and that the regulator protein could not be synthesized in the presence of chloramphenicol. We have reinvestigated this phenomenon and have shown that although RNA accumulation eventually halts after treatment with chloramphenicol, RNA synthesis continues. The failure to accumulate RNA was shown to result from a rapid degradation not only of RNA synthesized in the presence of the antibiotic but also of RNA that preexisted the challenge. Furthermore, both the rates of ribosomal and transfer RNA syntheses are vitually unchanged by the presence of the drug. The presence of chloramphenicol does not affect the synthesis or regulation of RNA but only the stability of the nucleic acid.

2. Rate of assembly of RNA: The overall rate of RNA synthesis can be expressed as the product of the number of RNA chains under construction at any time and the average rate at which nucleotide residues are added to a single growing chain. Control of the rate of RNA synthesis could conceivably be mediated by mechanisms which alter the steady state number of RNA chains under construction, the rate at which these chains are elongated, or both. To decide between these alternatives we have developed a method for determining the steady state number of RNA chains under construction in cells during balanced growth. The second parameter affecting the rate of RNA synthesis, the rate of chain elongation, is obtained by dividing the total rate of RNA synthesis by the number of growing chains.

Using this analysis, we have demonstrated that the increase in the rate of RNA synthesis at faster growth rates is due solely to an increase in the number of chains under construction and that the rate of RNA chain elongation does not vary with growth rate. Furthermore, evidence has been obtained indicating that all RNA chains--messenger, ribosomal and transfer--are elongated at the same rate, 50 nucleotides per second at 37°C. This remarkably slow rate of elongation requires that each gene specific for the 23S ribosomal RNA is simultaneously transcribed by many RNA polymerase enzymes in order to account for the large amount of ribosomal RNA synthesized by cells. Two models can be proposed in which ribosomal RNA genes are transcribed tandemly by many polymerase molecules and in which the number of polymerase molecules per cell (and therefore the number of growing 23S RNA chains per cell) increase with increasing growth rate. In the first, the actual number of polymerase molecules per gene increases at faster growth rates, and affords a faster rate of RNA synthesis. In the second, the number of genes specific for ribosomal RNA increases at faster growth rates.

In the latter case it is conceivable that the number of polymerase molecules per gene is constant at all growth rates. The work described in the next sections distinguishes between these alternatives.

3. Quantitation of genes specific for ribosomal RNA: Preferential gene duplication under conditions of rapid growth has been demonstrated by others for those genes lying near the origin of DNA duplication. Since DNA duplication starts at a fixed point on the chromosome and proceeds only in one direction, genes near the origin are duplicated first and occur at a higher dosage than do those near the terminus. At first growth rates a new round of duplication can commence before the previous one is complete, yielding a multiforked structure in which the dosage of genes near the origin may be as high as four times that observed in the nonduplicating chromosome. We have examined the possibility that the genes specific for ribosomal RNA are clustered near the origin and that the dosage of these genes increases at fast growth rates. For this we employed the RNA-DNA hybridization technique and have measured the percentage of DNA that is complementary to 23S ribosomal RNA. This percentage, 0.24%, was the same whether DNA from rapidly growing cells or slow growing cells was isolated from slow growing or rapidly growing cells. However, only 0.11% of the resting (nonduplicating) DNA was complementary to 23S RNA. These results suggest that a) there are approximately four 23S RNA genes per genome, b) that these are clustered near the origin of duplication, c) that the multifork structure of DNA occurs even at relatively slow growth rates (but that the gene dosage does not significantly increase at faster growth rates). Since the dosage of ribosomal RNA genes does not increase with growth rate, only the first of the two models proposed in the previous section is consistent with available data.

Significance to Bio-medical Research and the Program of the Institute: Control of nucleic acid synthesis in bacterial systems represents examples of regulatory mechanisms which are at this juncture more amenable to experimental investigation than the more specialized mammalian systems. It is anticipated that information obtained from these simpler systems will help form a basis upon which to investigate the more complex regulatory mechanism operative in higher organisms and eventually to understand and control disease states of these systems.

Proposed Course of Project: 1. To investigate the parameters controlling the rate of RNA synthesis under conditions of restricted growth (amino acid deprivation, inhibition by drugs, etc.). 2. To study the co-ordinacy of the control of RNA synthesis. 3. To study the mechanism by which the number of growing RNA chains is regulated.

Honors and Awards: None

Publications:

Lazzarini, R. A., and Santangelo, E.: The effect of chloramphenicol on the rate of synthesis and stability of RNA in Bacillus subtilis. J. Bact. 95: 1212-1220, April 1968.

Serial No. NDB(I)-65 LMB/OC 1244
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Control Mechanisms and Differentiation

Previous serial number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: L. Robert Berberich, M.D., Peter Fortnagel, Ph.D. (Guest Worker), Walther Klofat, Ph.D. (Guest Worker), Rüdiger Schmitt, Ph.D., and Richard Wax, Ph.D.

Cooperating Units: Section on Chemistry, Laboratory of Metabolism, NHI

Man Years

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|---|---|
| Total: | 4.9 |
| Professional: | 2.9 |
| Other: | 2.0 |

Project Description

Objectives: Differentiation in higher organisms comes about by a multitude of individual biochemical reactions, each of which is subject to a specific control mechanism. In order to learn the major principles, we are studying such control mechanisms in simple biological systems in which biochemical and genetic methods can be combined. We have investigated three aspects which will be described: 1. The control of enzyme synthesis. 2. Control of sporulation. 3. Control of germination.

Methods Employed: The concentration and induction of α-D-galactosidase was measured by the liberation of p-nitrophenol galactoside. The mutational sites were genetically mapped by bacterial recombination and by phage P1 transduction. Mutants producing large quantities of dihydrofolate reductase were isolated by their resistance to trimethaprim, which is a specific inhibitor of this enzyme. Mutations in the different bacteria used were induced by Cobalt$^{60}$ or ethylmethanesulfamate. The incorporation of uracil-C$^{14}$ into RNA was measured by collecting bacteria on Millipore filters and immersing them in Bray's scintillation fluid. The dissimilation of glutamate-C$^{14}$ was observed by thin-layer chromatography and the citric acid enzymes were measured by the reduction or oxidation of NAD spectrophotometrically.

Major Findings: 1. Coordinate induction of α-galactosidase and thiomethyl-galactoside permease. Three types of mutants have been derived from Escherichia coli K-12 which were unable to metabolize the α-D-galactoside, melibiose. One type lacked α-galactosidase, another lacked the specific transport system termed thiomethyl-galactoside permease II. The third lacked both of these functions. The mutational sites were genetically mapped by bacterial recombination and phage transduction. All mutant types mapped in a cluster near the methionine A marker on the E. coli chromosome. Studies on enzyme induction revealed that the three α-D-galactosides, melibiose, melibiitol, and galactinol, induced α-galactosidase and permease coordinately; D-galactose also induced these enzymes, but only in a galactokinaseless mutant. The data indicate that α-galactosidase and permease II are part of a common operon.

2. Elevation of rate of synthesis of dihydrofolate reductase as a result of alteration in the protein structure. Mutants of Salmonella typhimurium resistant to trimethaprim have been isolated. Trimethaprim is a specific and potent inhibitor of dihydrofolate reductase in both mammals and bacteria. 19 resistant mutants were found in which the dihydrofolate reductase activity was increased 20 to 60-fold, as compared with the parent type. The pH optima and heat stability of these mutant proteins have been compared to those of a 40-fold purified preparation of the parent enzyme. One mutant enzyme displayed a shift in its pH optimum, whereas another had a reduced stability at 60°, both indicating that the mutation leading to increased enzyme activity is due to a change in the primary structure of the enzyme protein. Drug-binding studies making use of the fact that trimethaprim binds stoichiometrically to dihydrofolate reductase indicated that the increased specific activity in the mutants is due to an increase in the molar amount of enzyme protein rather than an improvement in the active site. Mutants resistant to increasing concentrations of trimethaprim have shown as much as a 1,000-fold increase in specific activity of dihydrofolate reductase. Preliminary genetic tests indicate that for the latter phenomenon two or more sites on the bacterial chromosome are responsible. The reasons for the control of this enzyme are unknown but intriguing. Current plans call for the precise genetic mapping for the 16 single-step mutants and the purification as well as the amino acid analysis of the mutant sequences in the altered enzymes.

3. Identification of biochemical lesions in strains unable to sporulate. Previous experiments have shown that sporulation mutants can be divided into two classes, those for which uracil incorporation into RNA can be elevated by any added carbon source and others for which only certain carbon sources give a response. Mutants of the latter type have been further investigated and found to be all blocked in different enzymes of the citric acid cycle. The location of this block was determined by measuring the dissimilation of glutamate-$C^{14}$ into different products identified by thin-layer chromatography. The results indicated which enzyme was blocked and the subsequent enzyme measurements verified this contention. Mutants blocked in aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinic dehydrogenase and fumarase have thus been identified. The method of glutamate dissimilation

measurements has also been used to identify the biochemical block caused by α-picolinic acid, which is a specific inhibitor of sporulation. This compound was found to inhibit aconitase, a ferrous metal ion-requiring enzyme. α-Picolinate is known to chelate ferrous ions and so are other chelating compounds which were subsequently found to inhibit aconitase both in vitro and in vivo.

Most surprising was the finding that mutants blocked in the citric acid cycle between α-ketoglutarate and malate did not require any additional compound for growth in glucose minimal medium. This portion of the citric acid cycle apparently is not required for vegetative growth but is indispensable for sporulation.

Most sporulation mutants could not be restored to sporulate by the addition of several carbon sources. One mutant, however, responded to such carbon sources, in particular to acetate or palmitate. In the presence of these compounds the mutant sporulated with nearly the same frequency as the normal strain. This mutant also produced a small amount of an antibiotic which is typically produced by Bacillus subtilis during sporulation. The small antibiotic production was correlated to a slow rate of sporulation. Since the nonresponding mutants, blocked outside the citric acid cycle, did not produce antibiotic activity, it is possible that the antibiotic production is necessary for sporulation.

4. Initiation of the germination of Bacillus subtilis spores by a combination of compounds in place of L-alanine. Spores of B. subtilis can be initiated to germinate by L-alanine alone. It has now been shown that this initiation can also be obtained by the combination of L-asparagine, fructose, glucose, and potassium. Asparagine and fructose undergo some reaction which subsequently causes the spores to respond to the addition of glucose and potassium much more rapidly than without the pretreatment. It has been shown that the compound accumulated during the pretreatment can be lost again during incubation in the absence of any added compound. The results prove that several enzymes must be present in spores which allow the metabolic conversion of added compounds to produce the substrates necessary for germination. The analysis of the involved enzymes is now feasible.

Significance to Bio-medical Research and the Program of the Institute: The molecular understanding of differentiation is more amenable to analysis in microorganisms than in higher organisms because of the speed of experimentation and the possibility to isolate and genetically characterize many mutants. The fundamental biochemical processes are essentially the same in both types of organisms. In particular, mutations specifically involved with differentiation can be found, the phenomenon of commitment of differentiation can be observed, sequential development of the formation of spores can be demonstrated, and the control of DNA, RNA, and protein synthesis are equivalent. Findings in microorganisms can therefore be extrapolated to postulates about the mechanisms of differentiation in more complex systems.

Proposed Course of Project: 1. Determine the mechanism by which elevated amounts of dihydrofolate reductase are produced in mutants which are altered in the enzyme protein itself. 2. Determine the mechanism by which sporulation is repressed during vegetative growth and commitment to sporulation is obtained once growth has stopped. 3. Analyze the importance of antibiotic formation for sporulation. 4. Determine the enzymatic blocks of sporulation mutants blocked outside the citric acid cycle.

Honors and Awards: None

Publications:

Freese, E., and Fortnagel, P.: Analysis of sporulation mutants. I. Response of uracil incorporation to carbon sources and other mutant properties. J. Bact. 94: 1957-1969, Dec. 1967.

Wax, R., and Freese, E.: Initiation of the germination of Bacillus subtilis spores by a combination of compounds in place of L-alanine. J. Bact. 95: 433-438, Feb. 1968.

Berberich, R., Kaback, M., and Freese, E.: D-amino acids as inducers of L-alanine dehydrogenase in Bacillus subtilis. J. Biol. Chem. 243: 1006-1011, March 1968.

Fortnagel, P., and Freese, E.: Analysis of sporulation mutants. II. Mutants blocked in the citric acid cycle. J. Bact. 95: 1431-1438, April 1968.

Serial No. NDB(I)-68 LMB/OC 1552
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Integrative Control of Macromolecular Synthesis

Previous Serial Number:

Principal Investigator: Michael Cashel, M.D., Ph.D.

Other Investigators: None

Cooperating Units: Department of Genetics, University of Washington, Seattle. Dr. Jonathan Gallant, Advisor to and co-investigator with M. Cashel during out-of-service training in genetics (1966-1967)

Man Years:

| | |
|---|---|
| Total: | 3.0 |
| Professional: | 2.5 |
| Other: | 0.5 |

Project Description:

Objectives: Under a given set of conditions, normal cells maintain balanced composition of their macromolecular components by integrative control of the formation of RNA, DNA, protein, and structural elements. In general, regulation might be exerted in three ways with mechanisms that affect: 1) the availability of low molecular weight precursors, 2) the polymerizing enzyme(s) which synthesize the macromolecule, and 3) the rate of breakdown of the component.

The dependence of RNA synthesis upon a complete array of amino acids is an exemplary regulatory phenomenon since this mechanism serves to integrate the formation of RNA with the synthesis of protein. The phenomenon is particularly amenable to study in Escherichia coli, since a large body of relevant physiological, biochemical, and genetic information is available. The aim of this investigation is to understand the mechanism by which amino acid availability regulates RNA synthesis.

Methods Employed: Measurement of RNA polymerase activity in whole cells is possible only under conditions which render the cells permeable to the phosphorylated substrates of the enzyme. This was accomplished by exposure of

cells to hypertonic sucrose solutions. Reaction conditions were derived which allowed the incorporation of isotopically labeled ribonucleoside triphosphates or their precursors into RNA. The production of nucleotides and phosphorylated metabolic intermediates in vivo was measured by thin-layer chromatographic analysis of acid extracts of cells labeled with $P^{32}$. In addition, conventional biochemical techniques were employed.

Major Findings: 1. Amino acid dependence of RNA polymerase activity. RNA polymerase activity, measured in sucrose-treated cells in the presence of all four ribonucleoside triphosphates, was only weakly amino acid-dependent in normal (stringent) strains as well as in mutant (relaxed) strains which overproduce RNA during amino acid starvation. In contrast, when labeled uracil, or uridine monophosphate (UMP) was substituted for uridine triphosphate (UTP) in identical reaction mixtures, incorporation of label into RNA showed a strong amino acid dependence in two stringent strains, but not in a relaxed strain derived from one of them. These observations indicate that the amino acid control mechanism is intact under the conditions of assay and that it acts at the level of generation of the pyrimidine triphosphate substrates of RNA polymerase rather than at the level of the enzyme itself.

2. Amino acid dependence of nucleoside triphosphate synthesis. The above suggestion was directly confirmed in the same cell system by measurements of the rates of phosphorylation of UMP itself. Amino acid starvation was observed to diminish the rate of conversion of UMP to UTP (as well as cytidine triphosphate) in stringent, but not relaxed strains. In cells whose permeability characteristics had not been altered with sucrose, all four ribonucleotide triphosphates were labeled with $P^{32}$ in the presence and absence of amino acids. These measurements indicate that amino acid starvation produces diminished rates of synthesis of purine as well as pyrimidine triphosphates. Thus the generation of all four substrates of RNA polymerase is amino acid dependent.

3. Relevance to amino acid control of RNA synthesis. It is likely, a priori, that amino acid control of the availability of the substrates of RNA polymerase might in turn control the rate of RNA synthesis. However, the results are also consistent with the hypothesis that the RNA control system acts directly upon the RNA polymerase reaction if the observations on triphosphate synthesis could be explained as a secondary effect of inhibition of the polymerase reaction. Accordingly, experiments were performed whereby RNA synthesis was inhibited by means other than amino acid starvation and the effects upon triphosphate synthesis measured. These results indicated that the polymerase reaction can be inhibited in any of three different ways (actinomycin, proflavine, or uracil starvation) without producing significant inhibition of triphosphate synthesis. Furthermore, when a stringent strain was grown on a limiting amount of a required amino acid in the presence of labeled uracil, an abrupt shrinkage of the pyrimidine triphosphate pools was observed to occur at the same time as the rate of RNA synthesis slowed due to amino acid exhaustion. Therefore the simplest interpretation is that amino acid starvation in stringent strains limits the availability of ribonucleoside triphosphates which in turn limits the rate of RNA synthesis.

4. The mechanism of amino acid control of ribonucleoside triphosphate synthesis. The impairment in the ability of stringent strains to phosphorylate mononucleotides after amino acid starvation could occur in two ways. These are involvement of regulation at the level of the phosphorylating enzyme itself, or at the level of availability of the high energy phosphate used in the phosphorylating reaction. It is not expected to affect de novo synthesis because of the results mentioned in part 2. In vitro enzyme studies have not so far revealed significant amino acid-dependent activities. Chromatography of phosphate-labeled cell extracts has revealed a number of intermediates in glucose fermentation pathways with strong amino acid dependence in stringent strains. Thus it appears likely that the amino acid starvation metabolic block is mediated by the availability of high energy phosphate. Localization of the particular amino acid-dependent reaction is being carried out by Dr. Gallant at the University of Washington.

Unusual nucleotides have been discovered whose behavior is so far consistent with the notion that they function as inhibitors of the amino acid-dependent phosphorylating reaction. The compounds appear upon amino acid starvation in only stringent strains. At 37° readdition of amino acids to cells results in the disappearance of the materials with a half-life of 10-20 seconds. The slowing of RNA synthesis due to amino acid starvation is not specific to starvation for any particular amino acid, nor is the appearance of the unusual nucleotides. Under all conditions the concentration of these compounds seems inversely correlated with the rate of RNA synthesis as well as the rate of synthesis of ribonucleotide triphosphates.

Significance to Bio-medical Research and the Program of the Institute: Amino acid control of RNA synthesis has been demonstrated in a variety of organisms possessing definite amino acid requirements. Integrative control processes are clearly a characteristic of all normal cells but are currently most amenable to analysis in microorganisms. Characterization of these processes is expected in general to lead to a better understanding of normal cellular physiology. In particular, problems concerned with RNA synthesis, protein synthesis, and energy control systems have recently proved themselves particularly relevant to neurochemistry.

Proposed Course of Project: To establish more rigorously the relevance of the unusual nucleotides to the inhibition of nucleotide phosphorylation and RNA control by amino acids, structural identifications, and examination of inhibitory functions in vitro.

Honors and Awards: Ph.D. (Genetics), University of Washington, Seattle, 1968.

Publications:

Gallant, J. A., and Cashel, M.: On the mechanism of amino acid control of ribonucleic acid synthesis. J. Molec. Biol. 25: 545-553, May 1967.

Cashel, M.: Studies on amino acid control of RNA biosynthesis. Ph.D. thesis. Department of Genetics, University of Washington, Seattle, 1968.

Cashel, M., and Gallant, J. A.: The control of RNA synthesis in Escherichia coli: amino acid dependence of the synthesis of the substrates of RNA polymerase. J. Molec. Biol. In press.

Annual Report of the
Laboratory of Neural Control, Intramural Research
National Institute of Neurological Diseases and Blindness

July 1, 1967 through June 30, 1968

Karl Frank, Ph. D., Chief

The Laboratory of Neural Control was officially established September 28, 1967. The Laboratory will begin work with a staff of five professionals in 3,000 square feet of space on the fifth floor of Building 36, hopefully, in the early summer of 1968. The work of the Laboratory will be a combination of basic and applied neurophysiology using a systems engineering approach. Its mission is three fold: 1) The development of principles and techniques by which information from the nervous system can be used to control external devices such as prostheses, communications equipment, teleoperators (devices for performing a mechanical operation at a distance). 2) The study of methods for control of the nervous system by forces outside the body--i.e., inward information transfer for the purpose of modifying the behavior of the nervous system. 3) The Laboratory will be engaged in basic research for the elaboration of mechanisms of control operating within the central nervous system.

Individual Project Reports will not be included in this Annual Report. However, initial research planned for the new Laboratory falls into several main projects intended to begin during Fiscal Year 1969.

## I. Chronic Multiple Electrodes

One of the first major objectives of the Laboratory will be the development of techniques for establishing a wide band connection (in the information sense) between the nervous system and external devices. Three possible approaches will be explored: a) Fine wire electrodes in contact with small bundles of fibers dissected from peripheral nerve or dorsal or ventral roots of the cat's spinal cord will be electrically isolated by a cast of silicone material and led to multi-contact connectors fastened to the spinal column or to transmitters for telemetering information outside the body. Such electrodes will be used both for recording of single unit impulses and for stimulation of single fibers or small groups of fibers. Preliminary experiments have already been carried out and show real promise for this technique. Improved resolution of single fiber activity may be possible through the use of pulse recognition techniques and manipulation of stimulus parameters. b) A second technique for making contact

between electrodes and single fibers or very small groups of fibers depends upon the ability of cut peripheral nerve to regenerate through small tubules containing metal or fluid electrodes. Preliminary work at The Johns Hopkins University indicates that peripheral nerve fibers will regenerate through the interstices in a teflon sponge and will at least enter the end of ten micron tubules. c) Electrodes rigidly mounted to the skull and projecting into the brain have been used chronically on both animals and man. However, such electrodes tend to produce lesions of increasing size with movements of the head and particularly with angular acceleration. The obvious solution is to use electrodes made of such fine flexible wire that they will move with the local brain tissue. However, such electrodes are generally not stiff enough to penetrate the brain. Preliminary tests are underway attempting to accelerate such fine wire electrodes to high enough velocity so that when they strike the surface of the nervous tissue they will penetrate a predetermined distance through their own momentum, rather like a straw in a hurricane which sometimes penetrates a board or tree. Alternatively, a pusher may be used to carry the flexible electrode to the desired recording or stimulation point -- the pusher would then be removed leaving the flexible electrode in place.

Special problems anticipated in the development of such chronic multiple electrode techniques are: The evaluation of electrode materials with regard to tissue reaction, electrical characteristics and mechanical properties; the theoretical and experimental study of potential and current fields surrounding single nerve fibers in peripheral nerve and the central nervous system; the development of special connectors and telemetering devices for conducting stimuli or impulses activity across the body wall; implantation techniques for providing the necessary electrical isolation of single elements; and the development of pulse wave form recognition and translation techniques such as voltage windows or other more elaborate methods.

II. Voluntary Control of Nerve Impulses

A second project will attempt to evaluate the possibility of establishing conditioning of single neural unit activity. Such experiments, using monkeys will attempt to determine the practical limits of individual proportional control of multiple neural elements, such as spinal motoneurons, pyramidal cells, etc. In studying the electrical fields associated with the establishment of conditioned reflexes, it is anticipated that adaptive filter techniques already developed by one of the future Laboratory staff will be found useful.

III. Analysis of Muscle Control

A third project will attempt to analyze muscle control loops in cats and monkeys. This research will attempt to describe the dynamic relationships among both sensory receptors and motor units responsible for skeletal muscle control. The project will necessarily include studies of basic mechanisms operating at the single cell level but will emphasize the multi-unit organization of motor control loops. Acute experiments will be used to gain information for chronic studies. Implanted strain gauges will be used to study the relationships in activity among various muscles in chronic preparations. A special muscle puller is under development which will permit preprogramming of the movement of a joint or muscle to follow a predetermined displacement or force schedule. Transfer functions will be studied for the various steps in muscle control feedback loops.

IV. Mathematical Analysis of Neural Control

A fourth project will attempt to apply information theory to neural control through a mathematical analysis of neural information, using time series analyses and other approaches. It is anticipated that the Laboratory of Applied Studies, in the Division of Computer Research and Technology, will establish a satellite computer unit in Building 36 adjacent to the Laboratory of Neural Control. Collaborative efforts are planned between the two laboratories, both for the mathematical analyses of neural information and for modeling studies of neural control. In addition, it is anticipated that on-line computer techniques will be used to translate neural motor activity into appropriate time-space patterns of stimuli delivered to sensory pathways during acute and chronic experiments on neural control.

A Technical Development Section within the Laboratory of Neural Control will eventually be established for the development of particular electronic and mechanical devices required by the Laboratory research objectives.

It is expected that extensive use will be made of the engineering expertise of outside laboratories and commercial organizations through the establishment of contracts for the development of specific techniques and devices. Members of the Laboratory of Neural Control will act as project officers on these contracts. One such contract with the Washington University School of Medicine for "Development of Instrumentation to Evaluate Sensory and Motor Impairment" has already been let.

The staff of the new Laboratory currently consists of

two neurophysiologists with training in physics and computer techniques and a secretary-administrative assistant. Three more neurophysiologists with background in mathematics, engineering and computer techniques will join the Laboratory early in Fiscal Year 1969, and it is hoped that in addition to two summer students one or more technicians can be added to the staff.

The Laboratory of Neural Control was established with the philosophy that while relatively little of the complex mechanisms operating in the nervous system have yet been understood there is already enough known to indicate that a concerted effort to apply some of this knowledge might be fruitful. The Laboratory hopes to move forward from classical studies of single nerve cell activity to the integrated multi-unit behavior which has meaning to the organism in a behavioral sense.

Finally, the Laboratory represents a frank attempt to create a truly interdisciplinary group where physiologists, engineers, physicists, and mathematicians, etc., work together as a team towards specific common goals dependent for their successful achievement on the contributions from all.

The future development of mankind may depend to a significant extent on the development of techniques for neural control. It is important, therefore, to know what possibilities exist for direct connection of the nervous system to the outside world.

Summary Report
July 1, 1967 through June 30, 1968
Laboratory of Perinatal Physiology
National Institute of Neurological Diseases and Blindness

Ronald E. Myers, Chief

The Laboratory of Perinatal Physiology in the last 3 years has reduced the numbers of people in various service and support categories and has attempted to increase the numbers of scientists functioning within the personnel allotted. Any such effort inevitably takes considerable time, but this effort will have been completed by the end of this year. In the spring of 1965 the Laboratory had a total of 6 scientists functioning in its several areas. Of course, the number of scientists had been kept down as a part of the shift in the directorship of the Laboratory at that time. At the present time the number of full-time scientists has increased to 14 with, in addition, 2 guest scientists, a third on sabbatical leave and a fourth on a post-doctoral fellowship. Beginning July 1, 1968, the number of full-time scientists should be 20, with, in addition, 4 and possibly 5 additional scientists, either on post-doctoral fellowships or on sabbatical leave. It must be emphasized that this increase in scientists has been made possible in large part on the basis of slimming in various aspects of the program. No new personnel have been made available to the Laboratory in the last 2 years. The central effort within the Laboratory Chief's Office is to maximize the scientific effort and to reduce to a minimum, in keeping with satisfactory functioning, various support and ancillary personnel.

However, the Laboratory of Perinatal Physiology in Puerto Rico represents a major effort of NINDB in a wide variety of areas of study in relation to nervous system. Because of its separation from the parent institution in Bethesda, it is necessary for the Laboratory to invest in a variety of functions normally not the responsibility of an Intramural Laboratory at NIH. It is necessary for the Laboratory from its own personnel to provide for a procurement section and a personnel section, the two together constituting the administrative support areas. In addition, the Laboratory maintains a large number of animals as part of its facility. This necessitates an animal care section, including veterinarian support. The Laboratory is in large part responsible for maintenance of facility and grounds. The Laboratory also maintains a guard service for the protection of the facility. Hence, in a general and overall sense, the Laboratory must maintain from its own allotted personnel

many activities taken for granted and centrally supplied at the National Institutes of Health.

The Laboratory includes in its activities major scientific efforts in the areas of primate ecology, social and reproductive behavior, physiological psychology, comparative neurology, and experimental neuropathology. Several scientists function in each of these above areas and the programs of the various sections can be described as follows:

Primate Ecology and Social and Reproductive Behavior Studies:

The activities of these two sections function in immediate relation to one another. To a good extent the personnel of the experimentally oriented section on Social and Reproductive Behavior also function on Cayo Santiago. Five investigators currently use the facilities of Cayo Santiago: two guest scientists and three staff members of the Laboratory. Two scientists work in Parguera. A group from the Department of Anthropology of Northwestern University has been intensively studying for the past eight years the behavior of all individuals within a social group of Cayo Santiago (Group F). This careful longitudinal study is based upon the belief that an intimate knowledge of the life history of individual animals in relation to others within the group represents one of the most important and fruitful steps in the direction of an understanding of the social dynamics and of the psychology of the rhesus monkey. Such an understanding, in turn, is centrally important to any efforts which may be directed to an experimental study of factors involved in personality development and of social interaction in a primate species, whether the effort is exploring the importance of experience in the development of personality or whether the effort attempts to determine the underlying neurological mechanisms. This careful long-term study of Group F has yielded many fruits in the way of an increasing understanding of the social dynamics of these animals. Present and past publications which have come out dealing with this and other work in the islands have included discussions of vocal communications of free-ranging rhesus monkeys, the determinants of dominance among free-ranging rhesus monkeys, daytime sleep patterns in a band of free-ranging rhesus monkeys, agonistic behavior of young rhesus monkeys following changes in social rank of their mothers, seasonal breeding of free-ranging rhesus monkeys, the development of social structure in free-ranging monkeys, interactions between free-ranging groups of rhesus monkeys, and the social behavior of long-term castrates in a free-ranging situation. Already completed and extensively described in the literature are studies of the population dynamics of free-ranging rhesus monkeys.

At the present time there are a number of studies under development concerned with the determination of degrees of social distance and social intimacy between different animals within two specific social bands of monkeys in the free-ranging circumstance. The degree of social distance and intimacy is, in this study, determined by physical separation between animals and also by their grooming interactions. It has already become clear that there is a marked tendency of monkeys to be associated in relation to kinship ties. Most closely linked by grooming and by physical closeness are mother-daughter pairs, second only to mother-infant relations. Close ties also occur between sisters, between cousins, and between aunts and nieces. The relations of male offspring to female relatives are complicated by the fact that males commonly leave their social band during early adult life to join another band. These studies clearly emphasize the matriarchal nature of monkey society and indicate the importance of ties of kinship in patterns of association. Studies are underway to determine the pattern of social interrelations between animals in two large social groups, one of which exhibits a high and the other a low degree of cohesiveness. Other studies are concerned with consort relationships between monkeys during the mating season in terms of identity of involved pairs. Studies are in process attempting to determine the occurrence and length of menstrual cycles using changes in sex skin coloration and in social behavior as indicators. There has been continuation of a sex skin coloration cyclicity in the female for a period of 2 to 3 months past the mating season. It has become clear that the hormonal status of the female is directly tied in with the coming birth of the rhesus monkey which can be predicted with a high degree of accuracy by mere observation of changes in the sex skin coloration. This finding is of considerable importance in tying down the factors involved in the onset of labor.

Considerable effort has been expended in characterizing the nature of interactions occurring between social groups of free-ranging rhesus monkeys. Particularly interesting has been the finding that the great majority of acts of social interaction between groups are carried out by peripheral males. Peripheral males are young adult males, generally between the ages of 4 to 7, who are peripherally located in the group and are of lesser importance in the dominance hierarchy. These peripheral males, to a good extent, withdraw from close bonds with their families and with their mothers and in about 50% to 60% of cases leave their own social groups to join other social groups. They typically require several years to enter into the mainstream of the activity of the group with which they are associated. Nonetheless, most exchanges between two social groups coming into contact occur between such animals. It is these selfsame

animals, the peripheral males, which have been shown by work on Cayo Santiago to be primarily involved with exploration of the environment and with the approach to and examination of novel objects inserted into the environment. It has been found that the older adult animals, the central animals within a group, are less exploratory and less directly involved with the more routine comings and goings within the monkey society. However, it has become clear that these more mature, centrally important animals, nonetheless, maintain a considerable vigilance and may utilize more peripheral animals as indicators of events and of interpretations of new circumstances that arise within the environment. In other words, the major animals observe lesser animals observing a circumstance and respond in relation to the response of the lesser animal. Studies are underway specifying the nature and the details of the close social ties between mother and infant.

The sleep patterns of the free-ranging rhesus monkeys both in Cayo Santiago and in Parguera are under investigation. The rhesus monkey spends short periods of time sleeping throughout the day. They sleep mainly from 10 a.m. to 3 p.m. when they gather together in small groups. Females sleep 11.3% of an hour on the average, but sleep seems most prominent during the hours from 10 a.m. to 1 p.m. when approximately 20% of the time is spent in sleeping. Males sleep only 2.4% of the time or significantly less than the female. Duration of a period of sleep, generally speaking, is less than 2 to 3 minutes; and the total time spent in sleeping is an aggregate of a multitude of short periods of sleep. Females sleep primarily while sitting on the ground, whereas males more often will be found to sleep in a lying posture. It is believed the longer period of sleep exhibited by the females may relate to the greater disturbance of nighttime sleep produced by their care of infants and their contiguity to infants and juveniles. The manner of sleep during the day may represent a way of gathering rest and at the same time maintaining vigilance.

Experimental studies have been underway within the experimental enclosures directed toward an observation and manipulation of the events and factors which act during the interaction between two social groups. Once more a predominant role was seen for the peripheral males in defining the nature of intergroup interaction. These studies have been most illuminating in indicating that such important facts as group dominance are curiously enough defined by the peripheral males rather than the central animals of the two groups. Dominance reversal of groups has been produced by the removal of key peripheral males of the dominant group. It has been determined that the interactions between alien

social groups, both in enclosures and in the wild, have been primarily antagonistic, although instances of play, presenting, following, and some social mountings have been observed.

It is of extreme importance to develop an understanding of the social structure, motivational factors, patterns of interaction, patterns of development of behavior, etc., with reference to the free-ranging unmolested monkey bands. Such studies in themselves advance our understanding in a fundamental way with regard to the general problems of social organization and social interrelationship and are of direct relevance to problems of human social organization and interrelationship. However, they are also centrally important to any effort to develop a program of study in relation to brain mechanisms that would purport to investigate the brain basis of the various manifestations of social behavior, personality development, and patterns of social interdependence. In efforts in the direction of the experimental analysis of social and reproductive behavior, programs are in early stages of development. It is anticipated within the next year that the important facility programmed for Sabana Seca will be completed and that vigorous studies will be developed in this area directed primarily at an understanding of experiential factors and of brain mechanisms underlying social behavior. Efforts already underway in this area include studies on the development or ontogeny of patterns of aggressive and submissive behavior in the rhesus monkey. The effort will concentrate on factors which may influence or condition their development including maternal dominance status, the status of other significant animals in the environment, and the relation to the peer group. It has already become clear from observations of the free-ranging animals that maternal and familial status is of major importance in defining the ultimate status of a specific developing infant. Earlier studies of the rhesus on Cayo Santiago and of the Japanese Macaca in Japan have indicated that animals who assume leadership roles within natural bands are offspring primarily of mothers who are of high rank in the social hierarchy.

Animals subjected to deprivation of important social experiences and social interaction during early development exhibit severe alterations in their expression of social behavior. For example, a recently completed study in the Laboratory has shown that maternal deprivation during infancy and childhood results in an inability to complete the reproductive act on the part of male rhesus monkeys. In a detailed study of maternally deprived and wild-reared rhesus monkeys,

significant decreases were found in the frequency of grooming of the female by the experientially deprived males. There were significant decreases also in the duration of grooming bouts of the inexperienced versus the normal male. There was a decrease in the total grooming performed by the female on the deprived male versus the normal. Female bouts of grooming of the deprived males were shorter in duration as well as less frequent. The experientially deprived males were significantly more aggressive towards the female than the normal male, the aggressive behavior representing a distortion and a socially maladaptive expression of the normal aggressive behavior of the rhesus monkey. In contrast, the normally reared male exhibited significantly more threat gestures directed toward the female than the experientially deprived male. These two facts taken together indicate that the threat, which is a socially conditioned response, can take the place of aggression, which is, from the point of view of species survival, a maladaptive response. Normally reared males exhibited significantly more mount attempts than the experientially deprived males. The deprived males throughout the study were never observed to execute a normal mount. The deprived animals exhibited a profusion of stereotyped behavior or motor patterns and were inappropriate in their general handling of the social context.

Studies are nearly completed of the effects of social deprivation on the maternal behavior of the rhesus monkey. Although the maternal behavior deficit is nowhere near as complete as that expressed in relation to male reproductive behavior, still a major impairment occurs. Wild-reared females groom their infants significantly more than do the experientially deprived mothers. The infants of wild-reared mothers more frequently leave the maternal breast and protective sphere to explore the environment than do the infants of experientially deprived animals. However, the length of such separation from the mother is significantly shorter for the infants of wild-reared mothers versus those of deprived mothers. The wild-reared mother transmits many more return signals to the infant than does the deprived mother. The deprived mothers exhibit far more aggressive behavior toward their infants than do the wild-reared mothers. The deprived mother, in summary, although able to nurse and protect the infant to a degree, nonetheless, is a much less satisfactory and supportive mother than the normally reared mother. In like manner, the offspring of the deprived mother exhibits a great deal higher level of anxiety and inappropriate response than does the offspring of a wild-reared mother. Studies are currently underway to determine whether the effects of early experiential deprivation can be reversed by later exposure to a social situation.

One of the major concerns of the Laboratory is to study the neurological basis for the patterns of social interaction and social behavior which are under such intensive study both in the field and within experimental enclosures. Such efforts are already underway and have proven quite fruitful and interesting. At the present, little is known about the neurological basis for patterns of social interaction and social behavior, traits of personality, etc. What little evidence there exists suggests the limbic system may be of central importance to the expression of these aspects of behavior. If the question is raised of what role the cortex may play in defining patterns of social behavior, the answer is not clear. It may be that the cingulate cortex in conjunction with prefrontal and anterior temporal cortex may be centrally important for manifestations of social and emotional behavior. The first steps in analyzing which brain mechanisms contribute to social behavior have included studies involving the removal of the cortex of the anterior temporal lobe and in other animals of the prefrontal lobe. These removals were carried out on animals from one of the social groups of Cayo Santiago (Group E). On return to the free-ranging circumstance, these operated animals of both types remarkably failed to return to their social group and have, until this day, remained as solitary individuals on the island. This remarkable turn of events represents a major effect on the social behavior of these animals. Control animals of several types including sham operated have, on return to the island, rejoined their social group immediately and have exhibited no alteration in social behavior. At the present time efforts are being made to localize more closely within these larger cortical areas the mechanism sustaining social behavior and integration within the social group. Curiously, the anterior portion of the superior temporal gyrus, which anatomically is closely related to the prefrontal cortex, does not represent the critical locus for maintenance of normal interaction since complete lesions of this zone have failed to produce deficits in social behavior. In conjunction with a group from the Department of Psychiatry of the University of Illinois, a study is being instituted on the effects of amygdalectomy on social behavior in the free-ranging rhesus monkey. Particular attention will be paid to reproductive behavior since extensive alterations in this function have been seen in animals following amygdalectomy.

Studies on the neurological basis of reproductive behavior have already been initiated within the Laboratory using caged animals. The first step in this effort has been completed; specifically, that of describing the normative data illustrating the normal reproductive behavior of the caged male rhesus monkey. Grooming is most frequently

initiated by the female of the pair when the female is introduced into the male's cage. Approximately 70% of grooming is performed by the female. If episodes of grooming by the male and by the female are compared, the mean duration of grooming bouts performed by the female is considerably longer than those performed by the male. The male exhibits more grooming demands than does the female. The male in this circumstance carries out more instances of threat than he does of actual physical aggression against the female. Male aggression is preceded by failure of the female to respond appropriately to the male grooming demands or by failure of the female to assume the appropriate posture in relation to the male or a hyperactivity on the part of the female. The male typically executes a series of mounts leading to a consummation of the act. There is a period of rest followed by another series of mounts leading to ejaculation. Of particular importance is the fact that the free-ranging rhesus monkey exhibits a strict and marked seasonality in his reproductive behavior, going through a breeding season in the fall and a birth season in the spring. This is true both for the Cayo Santiago animals, for the animals in Parguera, and for monkeys in their natural habitat in India. However, in the caged circumstance within the Central Laboratory breeding behavior and births occur throughout the year. In the normative study carried out with the caged animal on reproductive behavior, it has become clear that 80% of all inactive matings with either no mountings or an incomplete series of mounts occurred during the months from November through May. Hence, there exists some reflection of a seasonality of breeding behavior also in the caged animal.

The study of the neurological basis of reproductive behavior was initiated following the careful development of normative data which occupied the Laboratory for approximately 2 years. Although early in its inception, this study has already revealed two interesting results. It has already been determined that removal of the anterior portion of the temporal cortex results in failure to return to social group of origin or to family and remaining solitary in the free-ranging circumstance. It was anticipated that the production of the same lesion in caged animals would result in serious consequences to reproductive behavior. However, to our great surprise, animals with such lesions failed to show any alteration in their pattern of reproductive behavior; furthermore, patterns of grooming and expressions of aggression, etc., also were not significantly affected. Hence, there appears to be a real enigma which requires further work in order to explain this remarkable difference between the effects of this lesion as produced in an animal in a free-ranging circumstance as opposed to its production in an animal in a restricted caged environment.

Animals have been subjected to bilateral removal of the amygdala in relation to reproductive behavior. This lesion was carried out in these animals because of the remarkable effect which has been described for amygdala removal in a variety of other species, including the rhesus monkey in earlier work by others. Again, to our surprise, this lesion did not produce measurable effects on the caged behavior of these animals. More specifically, animals with this lesion on first return to the cage fell entirely within their preoperative range in relation to both reproductive behavior and to other patterns of social interaction including grooming and patterns of aggression. However, on longer term survival post-operatively, the males with this lesion gradually developed some statistically significant increase in the frequency of mating. However, the extreme degrees of hypersexuality which has been described in the literature did not occur in these animals. These studies will be continued with a careful examination of various brain parts with regard to their importance to social behavior.

The dated breeding program of the rhesus monkey, which constitutes an aspect of the Laboratory of major importance, is undergoing a careful examination at the present time by the staff of the program under the direction of the veterinarian. This examination has essentially two goals; namely, increase in efficiency of breeding with an increase in total numbers of dated pregnancies per annum, and, secondly, investigation in a scientific fashion of various aspects of primate fertility and reproduction. Over the past year something close to 100 relatively infertile females have been removed from the breeding program and have been replaced by females brought in from the vendor. Through a combination of the removal of these relatively infertile females and the more careful surveillance of the breeding program, a significant increase in the number of pregnancies is anticipated over the coming year which should bring with it a significant increase in the progress of studies in the areas of perinatal brain damage and perinatal physiology.

A careful study has been made of the causes of infertility in these females who were culled from the program. A variety of pathology has been described, one of the most interesting having been that of endometriosis; but other lesions such as fibroids, polyps, etc., have been described and will be published in the near future.

Physiological Psychology:

Progress has been made in the analysis of the neural

mechanisms underlying perceptual and mnemonic functions. Of particular interest in this area have been continued studies of the functions of corpus callosum. The major dependency for the interhemispheric transmission of information relating to the skilled motor act, already demonstrated for the champanzee, has been confirmed by a more careful study in the monkey. More important, the relative contribution which vision may make to the solution of motor skills has been carefully investigated. Monkeys with total forebrain commissure section still exhibit the capability of transfer of learned motor skills between the hands in the presence of vision. Similar outcome obtains where the animals are able to view their initial problem solving through one hand even when such visual observation is denied on transfer testing through the untrained hand. Thus, the possibilities resolve into two -- there is either an incoding of memory effects and a capability of organization of motor response within the visual mechanism sufficient to mediate the performance of a learned motor task or, alternatively, there are well-established and multivaried associational systems between the visual mechanism and that of the somato-sensory system. Further studies in progress should distinguish between these two possibilities.

Studies on the cortical substratum of facial expression in the monkey near completion. These studies have clearly shown that major lesions involving cortex of the several separate lobes in different monkeys produce only minimal impairment in the control of facial expression. Only with lesions of temporal lobe and of prefrontal cortex has there been facial weakness, and then only of mild degree.

Work has continued on conditioned vocalization in the monkey. Earlier work within the Laboratory had indicated an unexpected and remarkable difficulty in bringing vocalization under instrumental control. In earlier aspects of the work food reward was used as the unconditioned stimulus. In more recent work shock avoidance has been used and this, likewise, has failed to produce a conditioned response using vocalization as the instrumental response. These unexpected results are leading to the conclusion that the monkey as a species may not have a clear-cut volitional control over vocalization, but, rather, vocalization may originate from portions of the nervous system reflective of emotional or affective states. Hence, vocalization in the monkey may be purely nonpropositional. Major lesions of cortex have failed to influence vocalization patterns in the monkeys. These results with vocalization and those above with facial movements suggest little importance of the cortex in the communication functions of the monkey.

Studies have been carried out on the functional role of the prefrontal cortex and of the cortex of the anterior portion of temporal lobe. These have suggested deficits in social behavior. These areas are under further investigation in the area of physiological psychology. It is known from the work of others that lesions of the prefrontal cortex result in severe deficits of delayed response performance, of delayed alternation, and of the visual go-no-go response. No studies of these functions have been carried out in relation to the anterior portion of temporal lobe. This is true despite the fact that the prefrontal cortex and the cortex of the anterior temporal lobe are interconnected through the uncinate fasciculus and that these areas share anatomical interrelationships with the dorsomedial nucleus of the thalamus. Present studies in the Laboratory indicate that removal of the cortex of the anterior portion of the temporal lobe results in a striking loss in the capability of either performing and of relearning the go-no-go performance. However, no effects could be seen on the performance of delayed response following these lesions. These animals with lesions of anterior temporal lobe were then tested on learning ability on visual color and visual pattern discrimination tasks where they showed some mild detriment in learning performances. On tests of response extinction, surprisingly, animals with the lesions of anterior temporal lobe required many more trials for extinction of the response than did the normal controls. These studies are being extended to effects of these lesions on the learning of tactual discrimination tasks in order to determine whether the deficit may be specifically a visual one as suggested by the visual losses or whether it may occur in relation to motivation or comparison behavior. Comparable studies have been carried out in animals with major lesions of the dorsomedial nucleus of the thalamus where, surprisingly, no deficits were seen on any of the tasks tested. This startling result indicates for the first time that lesions of thalamic nuclei do not necessarily duplicate the functional deficits found after lesions of related cortical areas.

Studies have continued on localization of functions of corpus callosum. There have been no studies of the possible functions of the anterior portions of the corpus callosum. This portion of the commissure is anatomically related to the prefrontal cortex and to the central gyrus. Since deficits of delayed response performance, of delayed alternation, and of go-no-go performance are found after lesions of prefrontal cortex, studies were done on commissure-intact and commissure-sectioned monkeys to determine whether corpus callosum is required for the transfer of these performances from one side of the body to the other. Results indicate that commissure section results in a complete interruption of or severe

disturbance of the transfer of these functions between the two sides. The deficit was more severe in relation to go-no-go performances than to the other two tasks tested.

Several studies have recently been undertaken using the pigeon for the study of the neural mechanisms underlying perception and memory. These studies look into the commissural basis for interocular transfer of visual learning in the pigeon and have suggested a dependency on the commissure of the tectum for this important process. Dependency for the transfer of complex patterned visual learning on the tectal commissure in the pigeon is in contrast to that circumstance in the cat, monkey, and chimpanzee where this commissure mediates only information handling regarding brightness, flicker frequency, and color vision. Thus, this evidence taken together supports the idea of an encephalization or a cerebralization of more complex perceptual and mnemonic functioning. In parallel with this study, an anatomical investigation of the posterior and tectal commissure is being carried out in the pigeon. This study has shown that the commissural connectionism through the tectal commissure is to a large extent restricted to the midbrain at its own level. There are minimal fiber projections passing more rostrally into the diencephalon. There are more prominent fiber pathways which lead more caudally into the intermediate portions of the tegmentum. The visual pattern learning transfer process in the bird would appear from these studies to be first and foremost a function of the tectum itself whatever the contribution of the midbrain to the process.

Comparative Neurology:

The work in the Comparative Neurology Section concerns itself with the structural analysis of the central nervous system of several carefully chosen species in each vertebrate class. These studies are carried out in an attempt to elucidate problems of human central nervous system organization by broadening our understanding of brain organization of lower vertebrate forms. Simpler brains of lower vertebrates present many fundamental features of the human brain without the numberless complications which obscure these fundamentals in higher forms. Studies on inter-neuronal connections have been emphasized in these studies. Current investigations result in new knowledge about the projections of the retina in several species of fish, amphibians, and reptiles. Ascending spinal projections and the afferent and efferent connections of the mesencephalon are also being studied in these forms. An investigation of the organization of the non-mammalian telencephalon ties together with the other studies in the

elucidation of inter-specific differences and similarities of organization. These studies are among the first comprehensive studies of their kind using the Nauta method. As progress in neurobiology has been closely related to the development of new methods, considerable effort has been made to improve old histological procedures and develop new ones. Such new techniques are already being employed in present comparative studies. Improved methods for neuromorphometric analysis are also being developed. These comparative neuroanatomical studies are in the preliminary stages but have already given many clues to the overall organization of the nervous system.

Experimental Neuropathology:

Studies have continued attempting to define the neuropathological implications of various adverse factors acting during pregnancy or at the time of birth. Progress has been made in studies on the neuropathological effects of umbilical cord compression at different gestational ages. Compression of the umbilical cord essentially produces a period of acute total asphyxia in the fetus. Such damaged fetuses may be returned to the uterus and brought to term. The pathological patterns produced in the brain at different gestational ages by this method resemble the patterns of pathology produced by asphyxia given at the time of birth. In both instances there is bilateral symmetrical damage to brainstem nuclear masses or brainstem loci. With umbilical cord compression, as with term asphyxia, there is a relative preservation of the hemispheral white matter and of the cortex. There are, however, important differences in the details of the pathological findings in asphyxiated fetuses of earlier gestational ages versus the later damaged fetus or the term infant. More specifically, the earlier the insult is sustained, the more relatively preserved or intact are the more rostral brainstem loci in relation to the degree of damage experienced by more caudal brainstem loci. Thus, in earlier developmental stages, there is a relative preservation of those portions of the brain which are relatively less mature at the time of insult. Spinal cord is relatively early involved in both the newborn baby and in fetuses subjected to umbilical cord compression at various gestational ages.

The physiological changes exhibited by the fetus during the course of umbilical cord compression compare closely with the changes exhibited in relation to asphyxia at the time of birth. However, the time rate of change of the alterations in blood chemistry and in cardiovascular physiology are less per unit time. That is to say, blood pressure is

better maintained in the earlier fetuses for longer periods of time as is the heart rate, electrocardiogram, etc. Also, careful studies of the lengths of umbilical cord compression required to produce first brain damage have clearly shown that the earlier the insult is delivered in development, the more refractory or tolerant is the nervous system to the asphyxial insult. This is a very significant effect, such that fetuses at approximately 100-110 days can tolerate up to twice the length of asphyxia as can a newborn baby prior to producing first evidence of brain damage.

In quite a different approach to the study of the factors producing damage to the fetus, experiments have been carried out in which the fetuses are removed from the uterus at different gestational ages, the carotid arteries and jugular veins ligated bilaterally in the neck, and the fetuses restored to the uterus. On surgical delivery at term these animals have shown a number of interesting findings. Approximately 45% of these babies do not survive the procedure in the first place but are aborted at some period of time after the procedure, usually within a week. Of those that survive one of three outcomes may be observed on examination of the brain: (1) The brain may show no evidence of morphological change as a consequence of the major vascular ligation in the neck. (2) The brain may exhibit severe destructive changes. (3) Finally, the brain may show no signs of focal brain lesions but may exhibit a variable degree of malformation of the cerebrum. Those brains which show no change in morphology, curiously, occur only in animals who sustained the vascular ligation in the neck at later times during gestation. Further, the data suggests that the later in gestation the vessels are ligated, the greater the probability of there being no change in brain morphology. Contrarywise, the earlier in pregnancy the vascular ligation is carried out the greater is the probability of cerebral malformation unassociated with evidence of discreet anoxic lesions. With animals who sustained ligation at 70 days or earlier, there is a high incidence of such severe cerebral malformation. Finally, cerebral destruction tends to occur in relation to ligation during intermediate stages of gestation. Usually the destructive change consists of total infarction in the region of supply of the anterior and middle cerebral arteries. In some instances the morphological change exactly replicates the pattern of pathology seen in hydranencephaly in the human. The earlier in pregnancy the ligation is carried out, the greater is the probability that the pattern of change, if destructive, will mimic hydranencephaly.

The particular pattern of cerebral malformation observed in these animals with vascular ligation in the neck

is of particular importance because of its close similarity to the pattern of pathology seen frequently in severe retardees usually sustained in state mental hospitals. Prior to the present study there has been no suspicion that this alteration in brain morphology had any relation to anoxic-ischemic insults. This was particularly so in view of the total absence of evidence for focal lesions in these brains. It now becomes clear that these altered brains almost certainly represent changes resulting from early insult to the brain of an anoxic-ischemic nature.

Studies have been completed on the effects of nembutal in protecting the brain against damage during the course of asphyxia. In this study, two series of animals were subjected to a specific asphyxial insult at the time of cesarean delivery at term. Those animals receiving barbiturate prior to delivery showed unequivocal and striking evidence of protection against brain damage compared to babies similarly delivered but under local anesthesia. It is believed that this significant and striking protective effect of barbiturate may be in relation to the lowering of the metabolism of the cerebral tissue.

In earlier progress reports, it has been described how magnificently close is the model of lead encephalopathy in the monkey in relation to that exhibited by the human. This was true not only for the clinical circumstance and the physical findings pursuant to lead encephalopathy but also the neuropathology itself was very closely similar. These uncompleted studies have again been picked up and will be carried to completion during the course of the coming year in order to fully establish the similarity of the changes in the monkey and in the human. The relative efficacy of various modes of treatment will be examined in relation to this model.

The neuropathology of acute total asphyxia has been described above. It was emphasized that the pattern of pathology in this circumstance encompasses specific loci in the brainstem. Curiously, this pattern of pathology bears little resemblance to the various well-recognized patterns of pathological changes in the brain seen in humans who are afflicted with cerebral palsy or mental retardation on the basis of perinatal brain damage. This important and central discrepancy between the findings in the monkey and in the human remained a frustrating and disturbing enigma until more recent times when further studies have brought to light the circumstances under which the typical patterns of human brain pathology may be produced in the monkey. When monkey term fetuses were subjected to prolonged partial hypoxia instead of acute total anoxia evidence of brain swelling was seen for

the first time. Brain swelling has never been seen in relation to acute total asphyxia in the monkey. This already indicated a critical difference between the two degrees of oxygen deprivation. It was not long before many cases of brain swelling associated with varying degrees of cortical necrosis were seen. Sometimes the cortical necrosis involved only specific portions of the hemispheres usually symmetrically and sometimes both hemispheres would be entirely necrotic. Sometimes the necrosis would occur in isolation and sometimes it would be associated with hemorrhage. In cases of lesser degrees of insult with chronic prolonged hypoxia it was found that the animals may survive. Under these circumstances, the patterns of pathology seen on sacrifice of the animals later consisted of ulegyria (also called atrophic cortical sclerosis or lobar atrophy) sometimes associated with status marmoratus of the basal ganglia. Most importantly, these two patterns of pathology, ulegyria and status marmoratus, represent the classically recognized and the common patterns of change seen in the human brain of individuals damaged at the time of birth or during gestation. Hence, failure in the past to produce acceptable models of human perinatal brain damage in the monkey has related to the nature of the insult delivered -- that given to the monkey rarely occurring in the human circumstance.

It is of importance to recognize that the prolonged hypoxia is a naturally occurring circumstance of the fetus in utero when adverse circumstances of a wide variety of types occur. In fact, generally speaking, the circumstance of fetal compromise consists essentially of a depression of partial pressures of oxygen in the blood (i.e., hypoxia), mixed metabolic and respiratory acidosis, and signs of progressive deterioration of the fetus. If the degree of compromise is severe enough, the baby will show signs of varying degrees of brain damage. The brain damage may, indeed, be extremely severe, resulting in cystic alteration of the brain. In this case one has an individual with severe neurological findings and amentia. In other instances there may be cerebral palsies of varying degrees of severity with or without mental retardation. Exactly comparable cases in rather comparable circumstances have now been seen in relation to the rhesus monkey as an experimental model.

Biochemical studies have been carried out following up the curious changes in PAS positive staining of the astrocytes of the newborn rhesus monkey following acute asphyxia (see progress reports of last year). A careful quantitative study has been carried out of glycogen content in ground up cerebral tissue and also in other important organs of the body in order to (1) study the changes in

glycogen storage and glycogen metabolism of the cerebral tissue, and (2) study similar changes in glycogen content in other bodily organs in order to investigate the carbohydrate metabolism of the body as a whole in relation to asphyxial insult. The findings are too detailed to cover here, but the most interesting and important finding is that there is a very significant increase in the content of glycogen in the cerebral tissue following acute total asphyxia which peaks at the 12th hour post-insult and then returns to normal levels by approximately 48 hours. Changes in glycogen content of other organs also are dramatic following the perinatal asphyxia. However, control values done in animals not asphyxiated but merely delivered and maintained indicate that considerable proportions of these dramatic changes in bodily organs but not in brain relate to the process of birth itself and have no relation to the asphyxia. However, additional increments of change in glycogen content in most organs have been found to relate to the asphyxia itself. An enigma exists in that vast quantities of glycogen are released from the liver following birth itself, with additional increments associated with asphyxia. Curiously, the same is true for most other organs, including voluntary muscle and the heart. The interesting and curious question is where do the breakdown products of this glycogen go. In no organ is there a concomitant increase in glycogen that would account for a storage in an alternate site. A similar study of glycogen storage is under progress in relation to juvenile monkeys undergoing circulatory arrest. Here, similar increments in glycogen content of brain tissue are seen; however, there is a prolongation of the elevation of glycogen beyond the length of time seen in the newborn with, however, peaking at approximately the same time, specifically, 12 hours following delivery of insult and resuscitation.

Studies on the neurological and neuropathological consequences of circulatory arrest in the adult rhesus monkey are nearing completion. The major findings in this study indicate that when the heart is properly protected from the anoxic insult by virtue of an intact cardio-pulmonary circuit, the nervous system and other organs of the body can sustain prolonged periods of circulatory arrest without major clinical or pathological changes. Lengths of time which can be sustained with relatively intactness of brain are up to 18-20 minutes. Acutely, that is, even in these animals, during the first several days of post-procedure survival, severe neurological findings, including opisthotonus, severe extensor hypertonus, blindness, severe sensory change, depressed consciousness, etc., are exhibited. However, typically, these severe findings resolve during the first several days and over a period of several weeks, in most instances, will disappear

completely unless the period of arrest exceeds 20 minutes. If permanent residua are seen, they usually consist of mild to moderate degrees of sensory loss over the extremities, associated with some clumsiness. Mild cerebellar signs with broad based gait and stance also may persist. Neuropathologically, animals following circulatory arrest exhibit, primarily, pathology in the brainstem with areas of predilection including the Purkinje cells of cerebellum, certain brainstem loci, including vestibular nuclei, the inferior colliculi, and certain thalamic nuclei, including portions of the ventral nucleus of the thalamus. These loci of damage, although resembling those seen in the fetus with acute total asphyxia, nonetheless show a greater variability in distribution. Cortex involvement and involvement of basal ganglia are unusual in these animals and are usually associated with a history of significant hypotension following the period of arrest.

The effects of prolonged hypoxia without hypercarbia are undergoing intensive study. One of the most interesting and outstanding patterns of pathology seen under these circumstances and seen rather frequently has been a pattern of symmetrical necrosis of the white matter of the two hemispheres with a greater involvement in the dorsal regions and a symmetrical necrosis of both portions of the globus pallidus bilaterally. The important factor in the production of this pattern of pathology appears to be a moderately severe hypoxia of a duration beyond approximately an hour associated with a moderate degree of metabolic acidosis. Studies will be carried out to determine the therapeutic efficacy of the treatment of the associated acidosis by bicarbonate.

Serial No. NDB(I) 65-LPP 1259
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neuropathological effects of umbilical cord compression.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

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|---|---|
| Total: | .7 |
| Professional: | .2 |
| Other: | .5 |

Project Description:

Objectives: To determine the pattern of clinical and neuropathological change produced by umbilical cord compression. To determine the length of cord compression required for production of neuropathological change at different gestational ages.

Methods Employed: Hysterotomy with sterile delivery of fetus; fetal electrocardiography; catheterization of fetal vessels with recording of blood pressure; blood gas and acid base studies of fetal blood; compression of umbilical cord; return of fetus to uterus; fetal survival in utero; c-section on 158th day; assessment of clinical status of infant; survival into juvenile aged; and finally, gross and neuropathological study of CNS.

Major Findings: Depending upon length of cord compression and gestational age a variety of neuropathological changes are seen. The most severe is advanced cystic degeneration of the nervous system with destruction of the neural parenchyma of brain and spinal cord. Surviving neurons are seen only in dorsal root ganglia, myenteric plexus of the gut, and bipolar cells of the retina. Such brains exhibit complete necrosis with connective tissue replacement of the cortex and the white matter of the hemispheres, coagulatory necrosis with small vessel thrombosis and without connective tissue replacement in thalamus and basal ganglia, and with destruction of the entire neural parenchyma without necrosis and with preservation of the astrocytic element in brainstem and spinal cord.

Such animals do not survive beyond birth. A lesser damage includes a clinical state compatible with survival through the newborn period but with evidence clinically for severe CNS involvement including severe changes in postural tone and in motor use. Neuropathologically, such animals exhibit severe destruction of neurons symmetrically throughout large portions of the tegmentum of the brainstem, destruction of entire complexes such as the superior olivary nuclei, the trigeminal sensory nuclei, vestibular nuclei, and inferior colliculi. These changes are associated with capillary proliferation, macrophagic response and astrocytosis. Still lesser degrees of damage produce a clinical status resembling spastic quadriparesis with a neuropathological picture associated with destructive changes similar to that just described but of lesser extent. Frequently such animals, despite severe destructive changes in brainstem, are found to lack pathological changes in the cerebrum itself. Finally, umbilical cord compression for as long as fifty minutes at earlier gestational ages and as long as twenty minutes at later gestational ages have resulted in no clinical abnormalities and no pathological changes in the brain. The tolerance to asphyxia, as determined by the length of asphyxia required for production of brain damage, has been plotted for fetuses of gestational ages from eighty days through one hundred and sixty days. The shape of the curve derived is logarithmic with fetuses of younger gestational ages exhibiting remarkably tolerant to asphyxia.

Significance: The present studies have clearly established the validity of using the fetal and newborn monkey as an experimental model for the study of perinatal brain damage in the human. To date each of the classical and better known patterns of neuropathological change found in the human in relation to asphyxial brain damage has been duplicated in monkey model. The study of the pathogenesis of these various patterns of brain pathology in the experimental animal should aid in the better understanding of the pathogenesis of cerebral palsy, mental retardation, and brain insult in general in the human.

Proposed Course of Project: More cases will be developed of cord compression in the experimental monkey in order to better establish the spectrum of pathological change produced under these circumstances. These studies also enable the development of information with regards to tolerance to asphyxia and the length of asphyxia required to produce the varying degrees of brain damage.

Publications:

Myers, R.E.: Patterns of perinatal brain damage in the monkey. In James, L. S., Myers, R. E. and Gaull, G. E. (Eds.): Brain Damage in the Fetus and Newborn from Hypoxia or Asphyxia. Columbus, Ohio, Ross Laboratories, 1967, pp. 17-21.

Serial No. NDB(I) 65-LPP 1261
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Superior Colliculus in the Goat.

Previous Serial Number: Same

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigator: Dr. Ronald E. Myers

Man Years:
Total: 0
Professional: 0
Other: 0

Project Description:

Work on this project has been postponed. It will be resumed in the coming year.

Serial No. NDB(I) 65-LPP 1262
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Reproductive Behavior of Caged Rhesus Monkeys.

Previous Serial Number: Same

Principal Investigators: Dr. Ronald E. Myers
Dr. Elizabeth A. Missakian

Other Investigators: Mr. Luis R. del Río

Cooperating Units: None

Man Years:

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|---|---|
| Total: | 2.2 |
| Professional: | 1.4 |
| Other: | .8 |

Project Description:

Objectives: 1) To obtain normative data on the reproductive behavior of captive rhesus monkeys. 2)To determine whether seasonality exists in the reproductive activity of the captive rhesus monkey.

Methods Employed: A group of 12 feral-reared male rhesus monkeys were observed. Each male was mated approximately every ten days with a sexually receptive female. The initial two hours of the mating was observed and the following behavioral responses were recorded: grooming, aggression, mount frequency, ejaculation frequency, inter-mount interval, inter-copulatory interval, mount duration, ejaculation time and mount attempt.

Major Findings: 1. Grooming: a) Grooming is most frequently initiated by the female. b) Approximately 70% of the grooming is performed by the female. c) The mean duration of grooming bouts performed by the female is longer than those performed by the male. d) The male executes more grooming demands than the female. 2. Aggression: a) The male executes more instances of threat than actual physical aggression. b) Male aggression is preceded by failure of the female to respond appropriately to a male grooming demand, failure of the female to assume the appropriate copulatory position, hyperactivity of the female. 3. Reproductive Activity: a) The frequency of ejaculations within a two-hour mating session ranges from one to four. b) In 76.65% of mating sessions, at least one ejaculation was recorded. 23.35% of all mating contained one series of mounts, 36.53% of all matings contained two series of

mounts. 61.17% of all matings contained three ejaculations. Only .60% of the total number of matings contained four ejaculations. c) The following quantitative differences exist in the mounting activity in successive series of mounts: successive mounting series contain a greater frequency of mounts, successive series contain an equal number of thrusts per mount, the recovery period between series becomes progressively longer through successive series. 4. Seasonality: a) Mounting activity and ejaculation has been observed throughout the year. b) Viable spermatozoa have been found in the vaginal lavages of females throughout the year. c) 80% of all inactive matings (matings containing either no mounts or no complete series of mounts) occurred during the months from November through May.

Significance: Data relevant to the specific mounting activity across series indicates that a model proposed by Beach for lower-order mammals is not applicable to the behavior of the rhesus monkey. The finding of periods during the year in which reproductive activity decreases in terms of mount frequency and ejaculation frequency suggests the operation of a suppressed seasonality among these monkeys. Further experimentation is necessary to establish the existence of such a phenomena.

Proposed Course of Project: Observations of the behavior of these twelve male monkeys have been completed. A manuscript describing this study is presently being prepared to submit for publication.

Publications: None

Serial No. NDB(I) 66-LPP 1384
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Localization of Learned Motor Skill Functions in Corpus Callosum in the Monkey.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Project discontinued. Professional time involved in other projects. No efforts have been expended on this project.

Serial No. NDB(I) 66-LPP 1385
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Pathology of the stillborn.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

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|---|---|
| Total: | .9 |
| Professional: | .1 |
| Other: | .8 |

Project Description:

Objective: Pathology of the stillborn monkey.

Methods Employed: All stillborn monkeys are subjected to postmortem examination to determine state of preservation of tissues and to describe the pathology of CNS.

Major Findings: The stillborn may be divided into fetuses with advanced tissue maceration with peeling of epidermis, liquefaction or softening of internal organs, and loss of normal tissue coloration and fetuses with preservation of intact gross morphology of internal organs. Intermediate circumstances are found, the most common being brain liquefaction with preservation of intact gross morphology of somatic organs. Several patterns of neuropathological change have been seen. Perhaps most common has been the occurrence of perivenular hemorrhages in the white matter of the hemispheres with predominance in the white matter of prefrontal lobe, the white matter of the posterior parietal lobe and extending into occipital lobe. White matter necrosis with liquefaction may occur. This pattern conforms with that seen in humans exhibiting periventricular leukomalacia. Laminar necrosis also is often seen which exhibits several types: 1.Universal gross disruptive laminar necrosis with greater predominance in the depths of sulci. 2.Laminar necrosis within the neuronal laminae of the hippocampi. 3.Laminar necrosis occurring in cerebellum alone. The third is most common. Laminal necrosis is associated with gross brain swelling and hemorrhagic retinopathy. In addition to these types of pathology, there is a third, observed frequently in the stillborn fetuses,

namely, severe brain swelling without evidence for the other pathology changes.

The state of preservation of CNS and somatic tissues bears little relation to length of postmortem survival in utero. Rather, the state of preservation of tissues and the degree of postmortem autolysis expresses appears more likely related to the rapidity of fetal death in utero (see studies on chemistry of necrobiosis).

Significance: Careful study of the stillborn monkey may give clues to the causes for and nature of processes leading to fetal demise. Study of the brains of these infants have resulted in recognition of several types of pathology seen in the human. The absence of data with regard to the circumstances of fetal compromise has prevented knowledge of pathogenesis of these lesions.

Proposed Course of Project: Postmortem studies of the stillborn fetuses will continue. An attempt will be made to correlate the facts known about the type of fetal death with patterns of pathology seen in the brains of animals falling within experimental procedures.

Publications: None.

Serial No. NDB(I) 67-LPP 1386
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Experimental placental insufficiency in the Rhesus monkey.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Harvey M. Shapiro
Dr. Donald Cheek

Cooperating Units: Department of Pediatrics, Johns Hopkins University.

Man Years:

| | |
|---|---|
| Total: | 1.2 |
| Professional: | .4 |
| Other: | .8 |

Project Description:

Objectives: 1. The effects of ligation of the fetal placental vessels upon placental morphology. 2. The relationship between placental insufficiency and fetal dismaturity. 3. The relation between placental insufficiency and brain damage.

Methods Employed: Monkeys at different gestational ages are subjected to hysterotomy. The fetal vessels bridging the space between the primary and the secondary placental cakes are identified and doubly ligated so that the secondary placenta is completely devascularized. Incisions are closed and the fetus brought to term. On cesarean section at term the gross and microscopic placental morphology is described. The infant is weighed at birth and its various organs weighed with precision after dissection. Weighed samples of the various internal organs, including muscle and bone, are sent to the Department of Pediatrics, Johns Hopkins Hospital, for chemical analysis. The placenta is sent to Sinai Hospital in Baltimore for morphological and histopathological description.

This year these studies have been extended to a large series of sheep in which instead of ligation of fetal vessels there has been removal of a varying proportions of the uterine horn containing the cotyledonous placenta. The total cotyledonous weight, as well as the weight of individual cotyledons, is recorded and compared to control data in order to approximate the percentage of placental deprivation accrued by the procedures. Again, the animals are

weighed along with organs at birth and analysis done on the tissues.

Major Findings: 1. Placental vessel ligation results in considerable alteration in the gross and microscopic morphology of the secondary placenta. These changes have been described in a paper submitted for publication.

2. Dismaturity of the babies was seen in many cases. The greatest "small-for-dateness" observed exhibited a birth weight of only 55% of average for the newborn Rhesus monkey. Other animals with lesser degrees of pathological change of the placenta showed lesser degrees of gross retardation. This study is still under progress and more precise quantitative data will be in the offing.

3. Only one animal of the series so far has exhibited neurological abnormalities which consisted of extensor hypertonus and disordered use of the hind extremities with some clumsiness in the hands. This animal, despite 6 months survival, failed to develop normal gait. On examination of the brain there was evidence for astrocytic scarring in the white matter in the predilection zones for perivenular hemorrhage and periventricular leukomalacia which included the white matter in the parasaggital region. In addition, the subependymal astrocytes exhibited a curious hypertrophy almost to the extent of producing a mass lesion in both occipital lobes with less striking such abnormalities throughout the third and lateral ventricles and in the aqueduct of sylvius. This animal failed to exhibit pathological changes in the predilection zones for damage in animals subjected to acute asphyxia.

4. At this date no results have been obtained in the rather major study undertaken with the sheep. These results will be forthcoming in the coming number of weeks.

Significance: The findings relate to the problems of fetal dismaturity, to placental pathology in relation to alteration of fetal circulation and to brain damage in the perinatal period.

Proposed Course of Project: More cases are required in the monkey to complete studies both in terms of collection of data on dismaturity of the fetus and particularly in relation to biochemical changes and changes in cell number in relation to these procedures.

Publications:

Myers, R. E., and Fujikura, T.: Placental changes after experimental abruptio and fetal vessel ligation of Rhesus monkey placenta. Amer. J. Obstet. Gynec., accepted for publication.

Serial No. NDB(I) 66-LPP 1387
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Experimental placental abruption in the rhesus monkey and its relation to brain damage.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 2.0
Professional: 1.6
Other: .4

Project Description:

Objectives: To develop a model of abruptio placenta in experimental animal and to study the resultant brain pathology.

Methods Employed: Monkeys at various gestational ages are subjected to hysterotomy. Abruption of varying proportions of the placenta is produced by finger dissection. Incisions are closed and the animal is brought to term. At term, placental specimens are carefully studied for morphological changes and the fetus evaluated clinically and pathologically for brain damage.

Major Findings: In the exploratory studies thus far completed, abruptions were so extensive as to be incompatible with fetal survival in utero: In the first four animals studied, abruption of forty to fifty per cent of the total placental surface was produced. Fetal deaths followed in all four instances.

Significance: The proportion of the total placental surface which must be abrupted to produce brain damage of various types or to produce demise of the fetus can be determined at different gestational ages by using the techniques explored in the present experiment. These studies are important to understanding of the problem of spontaneous placental abruption in relation to fetal mortality and morbidity.

Proposed Course of Project: During the past year the number of primate pregnancies available did not admit of further extension of these studies on experimental abruptio placenta. It is hoped during the coming year the number of pregnancies may be sufficient to admit further extension of these studies.

Publications: None.

Serial No. NDB(I) 66-LPP 1388
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Perinatal asphyxia in the monkey and its CNS consequences.

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Copperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.2 |
| Professional: | .4 |
| Other: | .8 |

Project Description:

Objectives: To determine the patterns of clinical alteration and neuropathological change produced by asphyxiation at the time of birth. To attempt to correlate the patterns of neuropathological change produced with the functional changes occurring in the fetus during asphyxiation.

Methods Employed: Monkey fetuses are delivered by cesarean section at term and subjected to asphyxiation prior to first gasp by the placement of a rubber sac over the head. The lengths of asphyxiation are of predetermined length or are determined by the degree of functional deterioration produced in the fetus. An effort was made to produce the maximum degree of damage compatible with survival. Studies are carried out on the cardiovascular, blood chemical (acid base status), blood gases, pH, lactate, etc. and respiratory changes of the fetus during and after asphyxiation. Resuscitation is carried out using positive pressure oxygen ventilation.

Major Findings: With the varying lengths of asphyxiation there were varying degrees of brain damage clinically and neuropathologically apparent. The most common clinical alteration was sensory loss in the extremities and over the head of varying degrees, alterations in sucking and swallowing, alterations in the voice, and varying degrees of ataxia and clumsiness. In some instances atrophic or traumatic sores occurred over the face and head. With more severe damage the animals exhibited changes in postural tone and changes in use of extremities with the most severe instances showing almost total loss of use of all four extremities and severe degrees of flexor hypertonus in all four extremities. Pathologically, destructive changes were found in nuclei of predilection in the brainstem and in posterior ventral and

lateral ventral nuclei of the thalamus and infrequently in putamen.

In the animals showing more severe involvement there was extension of damage to increasingly larger amounts of tegmentum of the midbrain, pons and medulla, with involvement also of trigeminal and facial motor nuclei, involvement of the intermediate and ventral portions of the gray matter of the spinal cord, particularly in the lumbar region, and also extension of the destructive process in the thalamus to include progressively larger portions until this structure was literally destroyed except for small nuclear masses medially located. In general, the type and extent of neuropathological alteration could be correlated with parameters of functional alteration in the status of the fetus occurring during the asphyxial process. For example, hemorrhagic changes within the distribution pattern occurred only in instances of profound and prolonged drop in blood pressure with restitution of cardiovascular system status and survival for a period of time. Less commonly, other patterns of neuropathological change were seen such as laminar necrosis, focal cortical atrophy with ulegyria, etc. These changes were seen respectively under circumstances of severe fetal acidosis and of elevated venous pressure in relation to the asphyxial process.

Significance: The intent is to reproduce the patterns of neuropathological change seen in cases of human cerebral palsy and mental retardation. To a good extent the study has been fruitful in this regard. More important still is the effort to determine the critical parameters involved in the functional deterioration of the fetus during asphyxial insult and to relate these changes to the patterns of neuropathological change produced. Finally, efforts are being made to determine techniques through which parameters of functional deterioration of the fetus may be corrected during the asphyxial insult.

Proposed Course of Project: We hope to extend the number of physiological and blood chemical changes which are measured in relation to the asphyxial process. Efforts will be made to artificially produce alterations in functional status of the various factors determined to be important in order to more clearly define the pathogenesis of brain damage.

Publications:

Myers, R. E.: The clinical and pathological effects of oxygen deprivation in the newborn rhesus monkey. In Adamsons, Karlis (ed.): Diagnosis and Treatment of Disorders Affecting the Intrauterine Patient". New York, Springer-Verlag, 1968.

Serial No. NDB(I) 66-LPP 1389
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Experimental hydranencephaly in the monkey.

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Karlis Adamsons, Jr.
Dr. L. Stanley James
Dr. Gerald F. Lucey
Dr. Philip Dodge

Cooperating Units: Columbia Presbyterian Medical Center, New York, New York
University of Vermont, Burlington, Vermont
Massachusetts General Hospital, Boston, Massachusetts.

Man Years:

| | |
|---|---|
| Total: | 1.1 |
| Professional: | .5 |
| Other: | .6 |

Project Description:

Objectives: To experimentally produce hydranencephaly in the monkey.

Methods Employed: One theory suggests that obstruction to blood flow in the major vessels of the neck plays a major role in the pathogenesis of hydranencephaly in the human. In the present study, monkey fetuses are surgically removed from the uterus, preserving umbilical circulation. The fetuses are subjected to bilateral ligation of carotid arteries and jugular veins. They are then replaced in the uterus and the incisions closed. On cesarean delivery later at term the fetuses are carefully evaluated for signs of neurological abnormalities. The brain specimens are removed and studied for pathological changes.

Major Findings: Bilateral ligation of the carotid artery in the neck at a variety of gestational ages results in no cerebral pathology in a majority of cases. Ligation of both the carotid artery and the jugular veins bilaterally results in a proportion of the cases in gross infarction in the region of supply of the anterior and middle cerebral arteries. In other instances abnormal development of gyri have been seen. The classic neuropathological picture of hydranencephaly has been produced in only one instance in an animal in which there had been bilateral ligation of both carotid arteries and jugular vein and, in addition, production of fetal anemia.

Significance: Knowledge of the mechanism of production of hydranencephaly in the monkey will greatly elucidate the pathogenesis of hydranencephaly in the human.

Proposed Course of Project: Further studies are required to determine the etiological factors underlying hydranencephaly. At present it appears some further insult is required beyond occlusion of carotid artery and jugular veins.

Publications:

Myers, R. E., Brain Pathology Following Fetal Vascular Occlusion to be published in American Journal of Ophthalmology 1968. This was an invited contribution to a symposium on developmental features presented to the Society for Ophthalmologic Research.

Serial No. NDB(I) 66-LPP 1390
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The effects of oxygen in high concentrations and vasopressor substances upon the fetus.

Previous Serial Number: Same

Principal Investigator: Dr. Karlis Adamsons, Jr.

Other Investigators: Dr. Ronald E. Myers, Dr. Ingemar Joelsson, Dr. Salha Daniel and Dr. Allen Hyman

Project Discontinued: Insufficient data for publication.

Serial No. NDB(I) 66-LPP 1392
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Nembutal prophylaxis of brain damage

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Geoffrey S. Dawes
Dr. Forrester Kochborn
Dr. Benjamin B. Ross
Dr. L. Stanley James
Dr. Salha Daniels

Cooperating Units: Nuffield Institute for Medical Research, Oxford, England
University of Oregon School of Medicine, Portland, Oregon
Columbia Presbyterian Medical Center, New York, New York

Man Years:

| | |
|---|---|
| Total: | 1.0 |
| Professional: | .2 |
| Other: | .8 |

Project Description:

Objectives: In prior studies within the Laboratory both local and general anesthetics were used for surgical delivery of the fetus. Animals delivered under nembutal were more tolerant of asphyxiation than were animals delivered under local in the length of asphyxia sustained prior to deterioration of functional indices of the fetus. It was not uncommon, for example, for fetuses delivered under nembutal to sustain up to fifty heart beats per minute for as long as thirty minutes of asphyxiation whereas animals delivered under local anesthesia regularly experienced cardiac standstill after only fifteen to twenty minutes of asphyxiation. The present study seeks to further investigate the significance of barbiturates in the prophylaxis of asphyxial brain damage.

Methods Employed: Two series of animals are delivered, one under local and one under nembutal anesthesia. The animals of both series are asphyxiated for a specified length of time, measurements of various functional parameters of the fetus being carried on during and after asphyxiation. The animals are resuscitated with partial pressure oxygen ventilation and observed clinically for the first two weeks of life. The animals are then sacrificed and studied neuropathologically.

Major Findings: With the animals asphyxiated twelve and a half minutes, all animals delivered under nembutal general anesthesia exhibited either lit-

tle or no evidence for clinical abnormalities and on neuropathological examination showed evidence for only very minimal or no pathological change in the brain. All animals delivered under local anesthesia exhibited evidence of clinical neurological deficit of moderate to severe degree and on pathological examination revealed marked pathological changes in the typical distribution within the brainstem. Other animals delivered under local anesthesia but nembutalized on delivery prior to asphyxiation responded in a fashion intermediate to those determined above. It is inferred that adverse factors act producing fetal deterioration in utero prior to delivery. This deteriorated status of the fetus on delivery under local anesthesia results in a higher incidence of brain damage and death when challenged with acute asphyxia. Nembutal somehow prevents the development of fetal compromise. In addition, nembutal anesthesia produces a lowering of the metabolism of CNS tissue of the fetus resulting in a mitigation of the effects of acute asphyxia as measured both clinically and neuropathologically.

Significance: These studies are important for the problem of parturition and brain damage in the human.

Proposed Course of Project: Similar studies will be carried out with one or other anesthetic agents in relation to acute asphyxia in the newborn period. The effort here will be to compare the prophylaxis of brain damage under acute asphyxia of barbiturate anesthesia in contrast to general anesthesia produced by other agents.

Publications: None.

Serial No. NDB(I) 66-LPP 1395
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Hyperbilirubinemia and Kernicterus in the Rhesus Monkey.

Previous Serial Number: Same

Principal Investigator: Dr. Jerold F. Lucey

Other Investigators: Dr. Ronald E. Myers
Dr. Mario Ferreiro-Serrano

Project discontinued: Insufficient dated pregnancies for completion.

Serial No. NDB(I) 66-LPP 1396
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neuropathology of Lead Encephalopathy in the Rhesus Monkey.

Previous Serial Number: Same

Principal Investigator: Dr. A. W. Brann, Jr.

Other Investigator: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total: .4
Professional: .2
Other: .2

Project Description:

Objectives: Study effects of chronic lead ingestation on central nervous system of the monkey.

Methods Employed: Monkeys less than one month old are started on lead chloride in milk and are continued on lead chloride with increasing dosage, attempting to stay under amount that will produce acute lead encephalopathy.

Major Findings: No major alterations in clinical state of nervous system except for an occasional episode of acute lead encephalopathy which subsided with no residual deficits with discontinuance of lead. No animals have been sacrificed as yet.

Significance: This study was initiated from the suspicion in man that some cases of mental retardation may be secondary to chronic lead ingestation unassociated with acute lead encephalopathy.

Proposed Course of Project: The study is being continued at least for a year with increasing amounts of lead chloride with the advancing age of the monkey, keeping short of the amount

that will produce acute lead encephalopathy. At that time animals will be sacrificed to study effects on central nervous system.

Publications: None

Serial No. NDB(I) 66-LPP 1397
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Comparative Studies of Ascending Spinal Projections

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Dr. Peter Sterling
Dr. David Winter
Dr. Boyd Campbell
Mr. Jorge Camuñas

Cooperating Units: Harvard University
Walter Reed Army Institute of Research
Center for Neural Sciences, Indiana University
Institute of Marine Biology, University of Puerto Rico.

Man Years:
Total .6
Professional: .2
Other: .4

Project Description:

Objectives: 1) To define patterns of central nervous system organization in vertebrates. 2) To make a survey of ascending spinal projections in several species from each vertebrate class.

Methods Employed: The animals are sacrificed 7 - 30 days after hemisection of the spinal cord, or ablation of the dorsal column nuclei. The brains are then processed according to the method of Nauta. The silver impregnated degenerating axons are identified and reconstructions are made of the course and termination of the degenerated fibers.

Major Findings: Earlier reports have dealt with the ascending spinal projections in the Tegu lizard and the frog. These studies revealed a component of the dorsal funiculus that projected directly to the cerebellum. Since there was no evidence for a spinocerebellar component in the dorsal funiculus of mammals, lesions were placed in the dorsal column nuclei of eight cats in order to explore this issue further. This study resulted in the discovery of a pathway from the nuclei gracilis and

cuneatus to the dorsal accessory olive (which in turn is known to project to the cerebellum). Specifically, degenerating fibers can be traced ventrally from the lesion as dorsal internal arcuate fibers. After crossing the midline, these fibers turn slightly rostrally and laterally before entering the inferior olivary nucleus between the various lamina of the nucleus. From here, fibers enter the dorsal accessory nucleus in which profuse pericellular terminal degeneration is observed. This fiber system is tentatively interpreted as a link between the dorsal columns and the climbing fiber afferents to the anterior lobe of the cerebellum, relaying specific information from mechano-receptors in the skin and joint receptors to the cerebellum.

Significance: Comparative studies such as these are carried out in attempts to elucidate problems of human central nervous system organization by broadening our understanding of brain organization of lower vertebrate forms. These studies are the first comprehensive studies of this kind using the Nauta method. The findings in the lizard and the frog, are, with few exceptions, similar to those observations made in all mammals so far examined. The discovery of the dorsal spinocerebellar tract in the dorsal funiculus may represent a more primitive arrangement. A phylogenetic trend in the spino-olivary systems appears at this stage to be related to the increased role of the dorsal funicular system and associated structures in higher primates. It is noteworthy that attempts to demonstrate the classical ventral spino-olivary tract (VSOT) in chimpanzee and man have remained unsuccessful, although the connection is quite conspicuous in the rhesus monkey and other mammals examined. If the dorsal spino-olivary system (DSOS) is found to be concerned with more specific information than the VSOT, then it would be reasonable to expect that the DSOS is the vehicle for the spinal input to the dorsal accessory olive in chimpanzee and man. In general, however, a greater number of species of each vertebrate class must be examined before the generality of these observations can be ascertained.

Proposed Course of the Project: 1) To examine representative species of fish, amphibians, and reptiles for variation in ascending spinal projections using the Nauta method. 2) To examine these tracts and related nuclei using micro-electrode techniques in order to determine what kind of information the various tracts carry.

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Ebbesson, S. O. E.: Ascending axon degeneration following hemisection of the spinal cord in the Tegu lizard (Tupinambis nigropunctatus). Brain Research, 5: 178-206, 1967.

Ebbesson, Sven O. E.: A connection between the dorsal column nuclei and the dorsal accessory olive. Brain Research. (In press).

Serial No. NDB(I) 66-LPP 1398
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Development of neurohistological methods

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Dr. Kalman Rubinson
Mrs. Michie Vane

Cooperating Units: Walter Reed Army Institute of Research

Man Years:
Total: .4
Professional: .2
Other: .2

Project Description:

Objectives: 1) To develop more reliable neurohistological methods for the demonstration of degenerating axons, boutons terminaux, and neurons undergoing retrograde chromatolysis. 2) To improve existing methods for the demonstration of these neuronal structures, and to adapt existing neurohistological techniques for brains of submammalian vertebrates.

Methods Employed: The animals are sacrificed 1-30 days after enucleation of one eye and the brains are fixed in different fixatives. Variants of the Nauta method for the selective silver impregnation of degenerating axons are systematically investigated for improvement of staining characteristics and dependability. Investigations are underway for the development of a modification of this technique for paraffin imbedded material. The feasibility of employing fluorescent dyes for the demonstration of degenerating neurons and axons is currently being investigated.

Major Findings: Modifications of the Nauta method have been developed for the central nervous system of fishes, amphibians, and reptiles. Specifically noteworthy is a simple modification of Nauta's "uranyl nitrate method". The modification involves leaving the histological sections in a 0.01% potassium permanganate solution overnight instead of in a 0.05% solution for a much shorter period (45 - 240 minutes). When the higher concentration is used much time is wasted performing trials which are not needed in the new procedure. The results with the weaker

solution are consistently excellent and the modification is now used routinely in all of the comparative neuroanatomical studies in the laboratory.

A method for the selective silver impregnation of degenerating fibers and terminals in paraffin imbedded material has been developed. The method has been successfully employed for amphibian, reptilian, and mammalian material and its potential is currently being examined for use on other tissues.

Significance: Progress in neurobiology has always been related to the development of new techniques. The introduction of the methods for the selective silver impregnation of degenerating axons by Nauta has greatly widened the scope of neuroanatomical research. The adaptation of the technique for studies of submammalian vertebrates has broadened the field of comparative neurology. The capriciousness of the method, however, necessitates improvement as well as development of new techniques for the demonstration of degenerating axons and axon terminals. The preliminary findings suggest the feasibility of staining these structures by other than silver stains, namely, organic dyes. Such techniques would, hopefully, be easier and more reliable.

The development of a method for the selective silver impregnation of degenerating fibers and terminals in paraffin imbedded material constitutes an important technical advance in several ways. The capriciousness of the Nauta method is to a considerable extent due to the variability of fixation times, and it is well known that staining characteristics of tissues left in formalin deteriorate with increased time in the fixative. It is anticipated that early paraffin imbedding will preserve the tissue and therefore provide more uniform and predictable silver impregnation. The paraffin method is, in addition, considerably simpler to execute and should therefore require less technician time.

Proposed Course of the Project: 1) To continue the systematic search for staining reactions that are selective for degenerating neurons and their processes. 2) To continue the improvement of existing methods.

Publications: None.

Serial No. NDB(I) 66-LPP 1399
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Comparative Studies of Retinal Projections

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Dr. Kalman Rubinson, Dr. John Ramsey, Dr. Boyd Campbell, Dr. William Hodos, Dr. Harvey Karten

Cooperating Units: Institute of Marine Biology, University of Puerto Rico.
Center for Neural Sciences, Indiana University Division of Neuropsychiatry.
Walter Reed Army Institute of Research
Department of Psychology, Massachusetts Institute of Technology.

Man Years:

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|---|---|
| Total: | .7 |
| Professional: | .3 |
| Other: | .4 |

Project Description:

Objectives: 1) To define patterns of central nervous system organization in primitive vertebrates. 2) To make a survey of the retinal connections with the diencephalon and mesencephalon in several species from each vertebrate class.

Methods Employed: Unilateral enucleations of an eye have been made in the following species: 1) Toadfish (Opsanus tau); 2) Frog (Rana catesbiana); 3) Tegu lizard (Tupinambis nigropunctatus); 4) Moray eel (Gymnothorax funebris); 5) Tiger shark (Galeocerdo cuvieri) and Nurse shark (Ginglymostoma cirratum). The animals are sacrificed 5 to 70 days after surgery and the brains are then processed according to the method of Nauta. The silver impregnated degenerated axons are identified and reconstructions are made of the course and termination of the degenerated fibers.

Major Findings: The comparative studies have been continued this year with a considerable amount of time spent on developing surgical

and histological procedures for elasmobranchs and teleosts. The retinal projections in two teleost species have been analyzed in detail. Nauta preparations from toad fish (Opsanus tau) and moray eel (Gymnothorax funebris) having survived unilateral eye enucleation for 7 - 28 days revealed totally crossed projections to: 1) a small magnocellular, and 2) a parvocellular nucleus of the hypothalamus; 3) a few neurons in the fasciculus medialis tractus opticus; 4) a dorsomedial preoptic nucleus; 5) a pretectal nucleus which divides into a dorsal and a ventral part with the formation of medial and lateral marginal optic tracts; 6) a nucleus corticalis; 7) a laminated lateral geniculate nucleus (which could not be identified in the moray eel), and 8) the optic tectum.

Significance: This study on the retinal projections represents the first successful adaptation of the Nauta Method to fish material. Behavioral and physiological studies of the teleost visual system have been confined almost exclusively to the optic tectum. The present findings suggest the need for additional studies encompassing various other structures receiving direct retinal projections.

The successful completion of the study of the retinal projections in the shark and teleost suggests further studies in these species, such as ascending spinal projections, and opens up a whole new area of investigation in comparative neurology. It is hoped that the potential in the Laboratory of Perinatal Physiology in Puerto Rico be fully exploited during the next couple of years with guest scientists conducting a wide variety of studies on the nervous system of these forms.

Proposed Course of the Project: 1) To examine representative species of fish, amphibians, and reptiles for variation in retinal projections using the Nauta method. 2) To examine these tracts and related nuclei using microelectrode techniques in order to determine what kind of information the various tracts carry.

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Ebbesson, S. O. E., and Ramsey, J. S.: The optic tracts in two species of sharks (Galeocerdo cuvieri and Ginglymostoma cirratum). Brain Research, 8: (36-53)1968.

Ebbesson, S. O. E.: Retinal projections in two teleost fishes (Opsanus tau and Gymnothorax funebris): An experimental study with silver impregnation methods. Brain, Behavior, and Evolution, (In press).

Serial No. NDB(I) 66-LPP 1400
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Functional properties of cells within the juxta-striate area 18 of the Rhesus monkey.

Previous Serial Number: Same

Principal Investigator: Dr. Eugene C. Crichlow

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: Department of Physiology, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico

Man Years:

| | |
|---|---|
| Total: | .3 |
| Professional: | .2 |
| Other: | .1 |

Project Description:

Objectives: Anatomical findings have revealed that juxta-striate area 18 receives connections from the immediate adjoining portions of the striate cortex (Area 17). Juxta-striate area 18 sends fibers via the splenium of the corpus callosum into the homologous locus of the contralateral juxta-striate area 18, the ipsilateral "fingers" of area 18 proper and the homologous loci of the contralateral area 18 fingers. In this investigation an attempt will be made to deduce the functional characteristics of cells of this area.

Methods Employed: Extra-cellular unit recordings and intra-cellular unit recordings, when possible, will be used.

Major Findings: This project has just begun (January 1967). Electrical stimulation of the optic nerve has been used in an attempt to localize cells within juxta-striate area 18. At present little success has been achieved in isolating cells which respond to ipsilateral and/or contralateral optic nerve stimulation.

Significance: The juxta-striate area 18 lies in relation anatomically with that portion of the striate cortex corresponding to the vertical meridian. Since it is only these adjoining areas of striate cortex with project to juxta-striate area 18, the functional characteristics of cells of this area are particularly interesting inasmuch as they appear to be the only

means of more directly conveying information from one striate cortex to another.

Proposed Course of Project: To continue with project as planned.

Publications: None.

Serial No. NDB (I) 66-LPP 1401
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemistry of Necrobiosis of Mouse Brain.

Previous Serial Number: Same

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: None

Cooperating Units: None

Project Discontinued: There is a lack of reproducibility of the preparation due in part to a lack of control over physiological parameters.

Serial No. NDB (I) 66-LPP 1402
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemistry of the Necrobiosis of the Cat Brain.

Previous Serial Number: Same

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigator: None

Cooperating Units: None

Project Discontinued: There is a lack of reproducibility of the preparation due in part to a lack of control over vital physiological parameters.

Serial No. NDB(I) 66-LPP 1403
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: A Study of Prefrontal Lobe and Related Structures. (Formerly "A Study of Prefrontal Lobe: Its Functions and the Structural Bases.")

Previous Serial Number: Same

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigator: Dr. Ronald E. Myers

Cooperative Units: None

Man Years:
Total: .9
Professional: .4
Other: .5

Project Description:

Objective: To investigate functional relations of the prefrontal lobe and related structures in the monkey by behavioral techniques, thus attempting to expand our knowledge regarding the functional boundaries of the macaque brain as contrasted to the anatomical ones.

Methods Employed: Nine monkeys with prior behavioral training experience were preoperatively trained on delayed response and go-no-go type visual color discrimination tasks. After the completion of the training, three monkeys underwent bilateral cortical ablation in the anterior one-third of the temporal neocortex, three others bilateral removal of the dorsomedial nuclei of the thalamus, and the remaining three served as normal controls.

Two weeks after the surgery they were retrained on the two tasks.

Following the completion of the retraining, they were further trained on simultaneous object color discrimination, pattern discrimination, pattern discrimination reversals, extinction tests, and position habit reversals.

Major Findings: Immediately after the surgery, the temporal operated animals appeared noticeably tamer than their preoperative days. Their appetites were lower than before, and two of them actually lost some weight. However, a few weeks later these effects disappeared almost completely.

Postoperative performance on delayed response showed that neither temporal nor thalamic removals had any effect on these tasks. However, on the go-no-go task, rather marked impairment in performance was shown by the temporal monkeys, whereas thalamic monkeys did not show any difference from the normal controls.

Postoperative learning of object color discrimination and pattern discrimination revealed that the temporal animals took more trials to reach a criterion and made more errors than the thalamic or the normal animals. The difference between the latter two groups was again nonexistent. The performance on pattern discrimination reversals again revealed the impairment by the temporal animals but not by the thalamic. Further, the result on this task suggested that the difficulty in the temporal animals lies in either reversing the association of reward values to a discriminandum, or in inhibiting a conditioned response to a stimulus, rather than in perceptual capacity.

The result of extinction tests revealed no difference among the three groups. But in position habit reversals the temporals were again inferior to the other two groups who did not show any difference between themselves.

Significance: If the impairment of performance shown by the temporal monkeys in some of the tasks described above may be interpreted as being due to impaired ability to inhibit certain response tendencies, it seems to fit rather well with previously available evidence.

Performance deficit in go-no-go type of tasks was reported to be shown by the monkeys with the orbitofrontal lesion. The orbitofrontal area is interconnected strongly with the anterior temporal area by the uncinate fasciculus.

However, the lack of differences among the groups in the result of extinction tests seems to suggest a problem to the above interpretation. On the other hand, it has not been established that behavioral extinction phenomenon is due to active inhibition of a response tendency by an organism. Clearly further research is needed to throw more light on the questions.

Proposed Course of Project: To continue by adding more experiments.

Publications: None.

Serial No. NDB(I) 66-LPP 1404
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effects of Brain Lesions on Conditioned Vocalization in Monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigator: Dr. Ronald E. Myers

Man Years:
Total: .2
Professional: .2
Other: .0

Project Description:

Objectives: To observe effects of brain lesions on conditioned vocalization from the standpoint of understanding the relationship between the CNS and speech phenomena.

Methods Employed: Monkeys are placed in an operant training situation to be conditioned to vocalize to escape or avoid electric shock from a grid floor in a cage. After the training is completed, they undergo brain surgery and changes in conditioned behavior is measured.

Major Findings: Since a very simple paradigm of conditioning with food reward, when applied to the conditioning of vocalization, failed, conditioned avoidance paradigm was attempted. The monkeys failed entirely to learn to avoid shocks by vocalizing, though one monkey showed an apparent increase of avoidance rate. The result of this particular monkey appeared more likely due to the increase in his random vocalization level rather than a conditioned one. Also, there was a definite impression that the vocal responses emitted when animals felt shock were a kind not amenable to volitional control.

Because of the failures to condition vocalization with this paradigm, the attempt was discontinued, and another approach is being attempted.

Significance: The problem of CNS control of vocalization in animals has been neglected. Human speech has been described as having propositional and emotional aspects, the former being more dependent on the cerebral cortex. It is interesting that the monkey whose vocalizations appear to be mainly of emotional origin seems not to depend on cortex for this expression. In this regard vocalizations in the monkey may be more like emotional speech in the human.

Proposed Course of the Project: To proceed according to the plan, and also to extend the study to the subcortical CNS structures.

Publications: None.

Serial No. NDB(I) 66-LPP 1405
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Lactate concentration in amniotic fluid.

**Previous Serial Number:** Same

**Principal Investigator:** Dr. Salha Daniel

**Other Investigators:** Dr. W. H. Niemann

**Project discontinued:** Investigators no longer in the laboratory.

Serial No. NDB(I) 66-LPP 1409
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Embryonic tooth development in the rhesus monkey.

Previous Serial Number: Same

Principal Investigator: Dr. Bertram S. Kraus

Other Investigators: Dr. Ronald E. Myers

Man Years:

| | |
|---|---|
| Total: | .5 |
| Professional | .1 |
| Other: | .4 |

Project Description:

Objectives: Significant numbers of upper and lower jaws of monkey fetuses of different gestational ages have been forwarded to the Cleft Palate Research Institute at the University of Pittsburgh where these specimens have been carefully worked up for the pattern of tooth development at different periods in the life of the fetus. An atlas is currently being prepared for this by that foundation and should be coming out in the not-too-distant future. These normative values are crucial to the development of experimental studies on factors which influence tooth development.

Methods Employed: Specimens of the jaws of animals who have undergone experimental manipulalions such as umbilical cord compression or chronic asphyxia have been sent to this foundation for a careful evaluation of aberrations in tooth development which may have been consequent to the experimental manipulation and the experimental insult.

Major Findings: This study has been most exciting in that the results have been quite positive with clearcut and definite abnormalities in tooth morphology and tooth development. The important thing about these findings is that various kinds of afflictions or influences which may adversely affect human development producing patterns of cerebral palsy and mental retardation are experimentally associated with abnormalities in tooth development and tooth morphology.

Significance: It is possible by considering tooth morphology to determine the time of the influence of the adverse factors and to have some idea of their nature.

Proposed Course of Project: Both normal specimens and experimentally manipulated specimens of various types will continue to be forwarded to this group in order to determine the implications on tooth development of the various adverse factors.

Publications: None.

Serial No. NDB (I) 66-LPP 1410
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 throught June 30, 1968

Project Title: Reproduction and behavior in the ecology of the Rhesus monkey.

Previous Serial Number: Same

Principal Investigator: Dr. John A. Morrison

Other Investigators: Dr. Emil W. Menzel, Jr.

Cooperating Units: Delta Regional Primate Research Center, Covington, La.

Man Years:

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 2.0 |
| Other: | 1.0 |

Project Description:

Objectives: 1. To transfer a social group of 82 Rhesus monkeys from Cayo Santiago to Desecheo Island and compare the behavior of the group observed before and after the transfer. 2. To grossly and histologically compare the gonads and accessory sex organs collected from ale Rhesus monkeys on Cayo Santiago during and between annual mating season to determine whether these organs change significantly in relation to the annual mating cycle. 3. To observe whether the mating season of the monkeys at Desecheo Island differs from that at Cayo Santiago.

Methods Employed: The group was observed intensely from January to June, 1966, then 56 members were trapped and sent to Desecheo Island on 1 July, after the end of the birth season. The 26 members remaining on Cayo Santiago were observed frequently to describe their new relationship with other groups, and the members at Desecheo have been observed whenever possible to note their reactions to the new environment. The two subgroups are to be reunited on Desecheo Island in April or May, 1967, after which their reactions to each other will be described. Autopsies are conducted on selected individuals to obtain gonads and accessory sex organs for study.

Major Findings: Cayo Santiago is a low, L-shaped island off eastern Puerto Rico. It receives 60-80 inches of rainfall annually, and is densely covered with trees and herbs locally characteristic of warm, humid area.

Commercial monkey chow was provided to provided to protect the vegetation

precipituous mountain peak emerging 200 m above the sea, 13 miles off Puerto Rico's west coast. It exceeds 600 acres in size. Rainfall approximates that of Cayo Santiago, but runoff and evaporation are greater and plant species are typical of drier climate, especially in that much cactus is present. It is uninhabited by humans but feral goats and housecats are present.

By mid-1966, Cayo Santiago supported 682 individually marked Rhesus monkeys in seven mutually antagonistic social groups numbering 221 (Group C), 194 (A), 78 (E), 82 (H), 58 (F), 26 (I), and 24 (J) members each, as arranged in the order of descending intergroup dominance.

Group H was selected for transfer to Desecheo Island, and by dividing it into two parts of 56 and 26 monkeys each and maintaining proportional representation of sex and age classes as best as difficult trapping conditions permitted, we could observe three situations for potential changes in behavior. The first situation was that the smaller part remaining on Cayo Santiago became numerically smaller than Group F and equivalent to Groups I and J, and new dominance relationships were possible. Secondly, the larger part sent to Desecheo Island abruptly encountered a different environment marked by:

a) approximately 15-times greater area,
b) absence of other monkey groups,
c) more rugged topography,
d) drier, warmer climate,
e) unaccustomed species of vegetation,
f) lack of supplemental food and drinking fountains, and
g) loss of one-third of the group, involving the separation of close relatives.

Thirdly, we hope to observe the reaction of the two subgroups to each other when reunited on Desecheo Island in the future when they may:

a) reunite into a single group again, or
b) remain as distinct, antagonistic groups, or
c) remain as separate groups with some exchange of individuals rejoining relatives.

The subgroup sent to Desecheo Island retained cohesion except that the lowest-ranking adult male became solitary upon release and disappeared 3 days later. The second-ranking male disappeared from the group after 4 days. The dominant male retained leadership. Related monkeys maintained close family associations. Juveniles separated from mothers yet at Cayo Santiago remained accepted by the group.

Daily activity at Cayo Santiago included much resting time when grooming, nursing, and juvenile play was common. Monkey chow was readily available from a feeder. Group movement was usually a constant, slow progression of brief individual moves in one direction. At Desecheo Island they made rapid marches often exceeding 200 m. Between moves they rested, groomed, nursed, but mainly they spread out to eat grass, berries, flowers, bark, and decayed

wood. Infants began eating solid food earlier than age-mates at Cayo Santiago. Group dispersion increased to more than double the former spread at Cayo Santiago.

All monkeys soon appeared constantly fatigued and thinner. Flight distance remained unchanged, less than 10m, and all seemed indifferent to humans. Formerly, the infants and juveniles played incessantly, but no play was seen at Desecheo Island. The increased travel was restricted to a narrow, circular route over 1 mile long, invariably followed whether going clockwise or counter-clockwise. The northern end was on the high, central part of the island where more time was spent in resting and feeding, possibly because of lower temperature and more abundant food.

Observations were made again August 5 to 7, 1966. The missing males were absent, yet, all others were indentified except four yearlings. All were now alert, wary, and flight distance exceeded 10 m until reduced with bananas. Although extremely thin they seemed strong, running and climbing rapidly without apparent stress. They continued to follow the same circular route in long rapid marches, spending more time in the low, southern end where new fruit and berries were more abundant. No play was seen. Although the mating season had commenced at Cayo Santiago, adults on Desecheo Island had not dveloped reddening of the sex skin.

A one-day visit was made on October 11, 1966. Flight distance had increased to $\geq$15m. Group dispersal exceeded 100 m, and several small subgroups were scattered widely. They did not exhibit the circular travel pattern, remaining in the head of the valley. Fortuitously, the subgroups reunited eventually near the observers, at which time flight distance became 10-20m. The former second-ranking male had returned to the group.

The monkeys appeared to have gained weight by October, 1966, and had smoother coats. No monkey had new wounds. Only 34 animals were identified, including 11 of the 13 infants, but several others were seen and 45 were believed present. The mating season at Cayo Santiago was at a peak in October; adults on Desecheo Island yet lacked sex skin coloration.

The last visit to Desecheo Island occurred February 16 to 20, 1967. Forty-two monkeys were found, fourteen were missing. The survivors are yet thin, but appear strong and vigorous. The group manifests strong cohesion with surviving family-group members yet closely associated and orphaned juveniles were still accepted in the group. A new behavioral pattern has emerged in which all sleep near a water hole at the south coast and range upward, together, to feed during the day. They may ascend to the top of the island where we observed them eating cactus flowers. They evidenced no fear of observers, flight distance was $\geq$10m. As before, their principal activity was food-seeking. Juveniles were preoccupied with food, and only one brief play bout was seen.

As in October, no new wounds were seen. The adults were commencing to redden in the facial and perineal sex-skin areas, but no mating was observed. At Cayo Santiago the birth season had commenced, but at Desecheo Island no

females appeared pregnant. Juveniles at Desecheo Island were more self-sufficient than age-mates at Cayo Santiago. They spent much time along seeking food and less time with their mothers.

The 26 members of the group remaining on Cayo maintained dominance over Groups F, I and J until July 22 when Group I displaced H at a feeder. Group F continued to be dominated by H until August 1, when Group F defeated H after much fighting for a feeder. Group J displaced H at a favored resting area on September 2. Group H has been subdominant to all others since then. Group F was expected to achieve dominance because it is twice as large as the remnant of H. Although similar in size to Group H, Groups I and J have adult males superior in number and experience to H males.

Before losing dominance on Cayo Santiago, Group H ranged over the large section of the island with members dispersed over an area of 30 to 50 m. They rested much in open areas of sparser vegetation. They held feeders for long periods, eating chow unhurriedly. Infants and juveniles played constantly. After losing cominance, Group H rarely exhibited aggression to other groups, retreating quickly when approached.

Travel distance and frequency of Group H on Cayo Santiago has been reduced. Group H now stays in a limited area of dense vegetation and moves only to seek food or escape another group. Group spread was less than 20 m until recently; it is now occasionally 20 to 30 m. Group H now devotes more time to food-seeking, either awaiting an opportunity to quickly grab chow from a feeder or spending more time eating natural vegetation. Play among juveniles declined.

Juveniles whose mothers are on Desecheo Island groom with each other or with other juveniles or with adult males.

Reproductive organs from two adult males were obtained near the end of the mating season in October and November. Others were collected in April, 1967, when gross testes had enlarged during the mating season. No analyses of the organs have been made yet.

There have been as yet no further observations made on this group of animals which have been transferred to Desecheo Island. However, in May, 1968, a further trip is planned for further observation on the behavioral and reproductive changes that the new and adverse environment has had on this social group of animals.

Publications: None

Serial No. NDB (I) 66-LPP 1411
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Social behavior in enclosed primate groups.

Previous Serial Number: Same

Principal Investigator: Dr. Halsey M. Marsden

Other Investigators: Dr. Stephen Vessey

Cooperating Units: None

Man Years:
Total: 3.75
Professional: 1.0
Other: 2.75

Project Description:

Objectives: To investigate the social behavior of enclosed populations of primates, principally Rhesus monkeys (Macaca mulatta) with emphasis on inter- and intra-group relations.

Methods Employed: Primarily direct observation of marked monkeys confined in enclosures on Cueva Island, La Parguera. Group structure is altered by removing and introducing animals.

Major Findings: Experimental manipulation of group structure in a small, enclosed band of Rhesus monkeys resulted in four changes in the adult-female dominance hierarchy. Offspring of these females changed rank directly with the mother. Concurrent data on social relations other than agonistic have not been analyzed completely but grooming patterns in particulare are expected to show significant alteration following rank changes among family groups.

A study has been completed involving observation of inter-group social interaction between two groups of Rhesus confined in two enclosures connected by a tunnel. Most intergroup behavior was agonistic. Intergroup play, presenting, following, and social mounting were observed. Grooming and copulatory behavior were absent between groups. Intergroup dominance was established. Peripheral and immature males were the most frequent participants in intergroup activity. A manuscript covering this initial, descriptive phase of the study is now in press.

A second study on intergroup social behavior has been completed. Both peripheral males were removed from the dominant group (Group I). In the absence of these two animals intergroup dominance reversed. This was first manifested in a reversal of direction of movement across the tunnel connecting the two home enclosures. Group II (original subordinate group) members began to move into Group I's enclosure. In observing the emergence of the new intergroup dominance relationship it was concluded that agonistic intergroup relations show a continuum from stalemate (reciprocal acts of aggression between fragments of opposing groups) through territoriality (defense of home enclosure) to complete dominance regardless of location (forcing the subordinate group to come together and retreat as a group.) Territorial defense was seen when (1) the dominant group penetrated too deeply into the subordinate's home enclosure (2) the subordinate group was placed or moved into the dominant's home enclosure. In these latter instances the dominant group quickly re-entered their own enclosure and forced the subordinate group to retreat to their own home enclosure. Intergroup dominance was again reversed (to its original state) when only one of the two peripheral males was returned to the original dominant group (Group I.) As a final test of the critical effect of the behavior of Group I's peripheral male on intergroup dominance, he was introduced to Group II following the removal of Group II's dominant male. Thus Group II gained the peripheral male from Group I (who became dominant with Group II) but lost their original dominant male. Under these conditions intergroup dominance again reversed, this time completely. With the peripheral male from Group I now leading (dominant in) Group II, Group II forced Group I to retreat as a group regardless of location in either enclosure.

Social behavior within an enclosed group of green monkeys (Cercopithecus aethiops): Data have not been completely analyzed on the study of general social behavior and group formation. However, it appears that behavior within the small, enclosed social group closely resembles behavior recently reported for the green monkey in its natural habitat in Africa.

With the birth of four new green monkey infants in the enclosures the infant development study continues in collaboration with Dr. Stephen Vessey. An important series of observations were made during this study involving the quantitative documentation of a spontaneous adoption of the second ranking female's infant by the top ranking female. This adoption was probably accelerated but not initiated by the death of the foster mother's own infant. The second ranking female had another infant during the year and raised it herself. The adopted infant is now spending more time with its genetic mother, probably as a result of the appearance of the new sibling. The foster mother has no new infant.

Proposed Course of the Project: Studies of intergroup relations within the two-tunnel-connected enclosures will be intensified and expanded. This has proven to be an excellent method for studying intergroup relations in detail. Currently, a study of the effect of intergroup conflict on aggression within a single group is underway. Future studies will include the effect of food deprivation on intergroup aggression and dominance. No further expansion of the existing program is contemplated.

Serial No. NDB (I) 66-LPP 1411

Publications: Marsden, H. M.: Agonistic behavior of young Rhesus monkeys following changes in social rank of their mothers. Animal Behaviour, 16: 38-44, 1968.

Marsden, H. H.: Behavior between two social groups of Rhesus monkeys with two tunnel-connected enclosures. Folia Primatologica, (in press).

Serial No. NDB (I) 66 LPP 1412
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Social behavior, reproduction and population dynamics of free-ranging Rhesus monkeys at La Parguera

Previous Serial Number: NDB(I) 59-LPP 603

Principal Investigator: Dr. Stephen H. Vessey

Other Investigators: Dr. Halsey M. Marsden

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 3.75 |
| Professional: | 1.0 |
| Other: | 2.75 |

Project Description:

Objectives: To investigate free-ranging and enclosed primates with emphasis on social behavior, reproduction and population dynamics.

Methods Employed: Observation of marked animals. Selected animals are captured and withheld for various lengths of time to determine their role in inter- and intragroup social behavior.

Major Findings:

La Parguera

Population dynamics: As of 1 March 1968 the Rhesus monkey population on La Cueva and Guayacan Islands has increased to 216, 21 of which are maintained in enclosures. Fifty-four births (seven in enclosures) occurred from April through August, 1967, with a peak in May and June. Thirty-two of 47 free-ranging infants survive to date, a mortality rate of 0.32. The total of 54 births was distributed among 60 sexually mature females, giving a birth rate of 0.90.

Social dynamics: Four heterosexual bands occupy La Cueva Island, consisting of 65, 47, 27, and 9 individuals. A single group of 39 monkeys occupies Guayacan. Five males and two females are solitary.

Moult: The pattern of moult in individual animals has been determined and

two years' data have been collected on the timing of onset and completion of moult in the population as a whole. Adult males begin to moult in May, several months after the conclusion of the mating season. Females and young begin in the summer, towards the end of the birth season. Lactating females moult later than nonlactating females. All animals are completely moulted by the beginning of the next mating season in the fall. These data are being compared with those collected by Morrison at Cayo Santiago as part of a joint study.

Intergroup relations: A descriptive, quantified account of group interactions has been completed. Removal of individuals and parts of groups will continue in order to determine which monkeys are responsible for a group's rank in the intergroup hierarchy.

Infant development: Nine free-ranging infants, born during the spring of 1967, are being studied for one year. A time sampling method is being used so that quantitative data can be collected on such behaviors as suckling, grooming, playing, and so forth. Similar work is being done on infants in enclosed groups of green monkeys and Rhesus monkeys in a joint study with Marsden.

Night-time behavior: An infrared telescope (sniperscope) has been modified for use in observing monkeys at night. At present quantitative data on intragroup movements and vocalizations are being collected, as well as locations of individuals within the group.

Proposed Course of the project: Major emphasis will be placed on completing the comparison of moult between La Parguera and Cayo Santiago, continuing the study of free-ranging infants, and obtaining further information on behavior of Rhesus monkeys at night.

Publications: Vandenbergh, J. G.: The development of social structure in free-ranging Rhesus monkeys. Behaviour. 29: 179-194, 1967.

Vessey, Stephen H.: Interactions between free-ranging groups of Rhesus monkeys. Folia Primatologica (in press).

Serial No. NDB(I) 67-LPP 1425
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The effects of interdepression stimulation on the information content of interhemispheric transfer.

Previous Serial Number: None

Principal Investigator: Dr. Bruno Kohn

Cooperating Units: None

Man Years:
Total: .4
Professional: .4
Other: -

Project Description:

Difficulties in procuring suitable animals prevented progress in this study. Such animals are now available and the project will be carried out as outlined previously.

Proposed Course of Project: To continue with project as planned.

Publications: None

Serial No. NDB(I)'67-LPP 1426
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Intermanual transfer of tactile discrimination learning in relation to the age at which commissure section occurs.

Previous Serial Number: None

Principal Investigator: Dr. Malcolm F. Piercy

Other Investigators: None

Cooperating Units: Department of Psychiatry, School of Medicine
University of Puerto Rico, San Juan, P. R.

Man Years:
Total: 0.4
Professional: 0.4
Other: -

Project Description:

Objectives: 1. To ascertain whether the age at which a monkey is submitted to section of the forebrain commissures affects the probability that unimanual tactile discrimination learning will transfer to the untrained hand. 2. To delimit some of the other factors which determine whether or not such intermanual transfer occurs after commissure section.

Methods Employed: Three groups of rhesus monkeys are used as experimental subjects: 1. animals aged about 2 years which underwent section of the corpus callosum and the anterior commissure at birth; 2. animals of about the same age that underwent section of either (2) the corpus callosum only or (b) the corpus callosum and the anterior commissure, a few weeks before training began. 3. An un-operated control group. All animals are trained to discriminate in the dark between two test objects, using one hand only. When training is complete the untrained hand is tested on the same task and the learning scores of the two hands compared. The design of the experiment brings under control and examines the effect of the following variables: 1. the age at which commissure section occurs; 2. the effect of sectioning the anterior commissure in addition to the corpus

callosum; 3. task difficulty; 4. degree of overtraining. All animals are trained with their preferred hand and tested with their non-preferred hand.

Major Findings: No significant difference was observed between the early operated and late operated animals with regard to transfer of training. In a number of circumstances, curiously, both groups of animals exhibited significant evidence for transfer of training.

Significance: It has often been suggested that early transection of the corpus callosum in the mammal or cases of agenesis of the corpus callosum may be associated with quite different implications in terms of functional deficits than comparable lesions carried out in later life. The present study offers no support for the suggestion that early transection of the corpus callosum (at birth) has any different functional implications than its transection later in life.

The occurrence of clearcut transfer of training between the hands of certain tactual tasks in the commissure sectioned animals raises important questions which are not fully answered by the present experiment. No clearcut explanation for the discrepancy of findings in this study with those reported by other investigators earlier can be found. It may be noted that the findings of the present experiment are in agreement with the findings of certain other authors.

Proposed Course of Project: To continue with project as planned.

Publications: None.

Serial No. NDB(I) 67-LPP 1427
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Changes in Oculomotor Activity Following Cerebral Lesions.

Previous Serial Number: None

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: Department of Psychiatry, School of Medicine
University of Puerto Rico, San Juan, P. R.

Man Years:

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.2 |
| Other: | - |

Project Description:

Objectives: This study aims at elucidating the roles of three cerebral areas in the control of eye movements: a) the frontal eyefields (area 8); b) the visual sector of the occipital and temporal lobes (areas 17, 18, 19, and the posterior parts of areas 20 and 21); c) the superior colliculi.

Methods Employed: Using rhesus monkeys, lesions are procured in the three areas described and in combinations of these areas. The early and later post-operative changes in eye movements are to be observed, recorded and analyzed. The means by which eye movements are provoked is to be systematically varied.

Major Findings: So far, four different neurosurgical procedures have been carried out in six animals and the post-operative changes are under investigation. Some fifteen or twenty further procedures are planned. In the present pilot stage of the investigation some early diminution of eye movements has been observed following occipito-temporal lesions except for movements which are visually triggered. The effect of collicular lesions is not clearcut owing to technical difficulties in procuring lesions restricted to the superior colliculi. These results have demonstrated the need for the electrical recording of eye

movements pre- and postoperatively and the means for effecting this is at present being planned.

Significance: Eye movements have been experimentally provoked by electrical stimulation within the cerebral areas at present under investigation. At the same time lesion work in monkeys has not so far convincingly demonstrated that lesions within these areas markedly impair the normal pattern of spontaneous or reflexly provoked eye movements. It is hypothesized for the purpose of the present investigation that eye movements which are provoked by visual stimulation are mediated by cerebral mechanisms which are in large measure anatomically distinct from those which sustain eye movements provoked by other modalities of stimulation and eye movements which depend on the animal's motivational status rather than on a specific exteroceptive stimulation.

Accordingly, importance is attached to a) the ablation of the whole of the cortical areas known to be involved in visual cognitive performance, b) the method by which eye movements are experimentally provoked, c) the study of the effects of combinations of lesions which, it is hypothesized, sustain different aspects of oculomotor control.

Proposed Course of Project: To continue with project as planned.

Publications: None.

Serial No. NDB (I) 67-LPP 1461
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Social behavior, reproduction, and population dynamics in free-ranging Rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. John A. Morrison

Other Investigators: Dr. Halsey H. Marsden
Dr. Stephen Vessey

Project Discontinued: Principal Investigator has left the laboratory.

Serial No. NDB (I) 67-LPP 1462
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Aggression in free-ranging Rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Andrew P. Wilson

Other Investigators: None

Cooperating Units: University of California, Berkeley, Calif.

Man Years:
Total: .9
Professional: .9
Other: .0

Project Description:

Objectives: 1. To describe aggression within and between groups, emphasizing fighting, agonistic displays, and the ontogeny of aggressive behavior. 2. To relate aggressive behavior to reproductive behavior, group size, time of day, location of encounter, weather, and food supply. Individual factors such as age, sex, dominance, position, kinship ties, and friendships between monkeys are considered.

Major Findings: Males are more aggressive than females. They are less likely to break and run in fights and suffer more wounds than females. Members of a dominant family usually win a fight with a subordinate family even if the subordinate family representatives are larger and older. Alliances between members of family groups are evident in intragroup fighting. Male alliances, as well as female family ties, allow the establishment of dominance relationships in which a physically superior monkey is dominated by a weaker monkey which is a member of a supportive alliance.

Intragroup fights between males during the breeding season lead to the breakdown of friendships and the expulsion of certain males from the group.

The most striking feature of aggressive behavior of Rhesus monkeys on Cayo Santiago is its close relationship to individual sex differences and reproductive behavior. Cayo Santiago presents a unique opportunity to study the relationship of sex hormones and social behavior in free-ranging monkeys because five male castrates, a possibly cryptorchid or intersex males, and two oophorectomized females live among the normal monkeys. Male castrates show less aggressive behavior and stronger female ties than normal males.

The oophorectomized females do not behave differently than normal females and have been observed in copulation during the mating season.

Publications: Wilson, A. P. and Vessey, S.H.: Behavior of free-ranging castrated Rhesus monkeys. Folia Primatologica (in press).

Serial No. NDB(I) 67-LPP 1463
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Temporal Lobe Connections in the Monkey.

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigator: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.0 |
| Professional: | 0.0 |
| Other: | 0.0 |

Project Description:

No work has been done on this project this year.

Serial No. NDB (I) 67-LPP 1464
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemistry of the recovering asphyxiated monkey brain.

Previous Serial Number: Same.

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. Ronald E. Myers
Dr. A. W. Brann
Dr. Ronald Di Giacomo

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.0 |
| Professional: | .4 |
| Other: | .6 |

Project Description:

Objectives: It is proposed to study the levels of various substances in the brain and other tissues of the monkey neonate recovering from acute asphyxia.

Methods Employed: Monkey fetuses were delivered under Nembutal anesthesia by Cesarean Section and asphyxiated for 2 minutes beyond its last gasp. (This procedure was performed by Dr. R. E. Myers, Dr. A. W. Brann and Dr. Ronald Di Giacomo). The neonates were sacrificed at birth (the control); immediately after asphyxia, or at varying intervals after resuscitation. Brain, heart, lungs, liver, kidney and muscle were rapidly excised from the anesthetized neonate and frozen in an isohexane isopentane mixture cooled to the temperature of liquid nitrogen. Glycogen levels of these tissue were determined.

Major Findings: The data is not complete; however, analysis of brain, heart, liver and kidney indicate some striking facts. The levels of glycogen in the tissue of the monkey neonate in mg per gram wet weight are: Liver 115, muscle 32.5, heart 9.7, kidney 1.1 and brain 0.57. At the end of the period of acute asphyxia brain glycogen were: -80%, heart -74%, kidney -47%, liver +19%. The brain rapidly recovers its glycogen and by 12 hours after the insult it contains 170% more glycogen than the control. The other organs recover their glycogen stores much more slowly. Liver glycogen remains low for same period of time remaining at -92% 9 hours after the insult. Control studies indicate

that the brain glycogen changes are directly related to the anoxic insult. The dramatic drop in glycogen content occurs also in unasphyxiated controls where at 12 hours after birth are at -60%.

Significance: The data being gathered should enable us to separate the chemical changes due to the trauma of the anoxic insult and the trauma due to the birth processes. It is too premature to speculate what the results might be. More data must be gathered, especially from normal animals to determine the changes that are normal and the changes that are due primarily to the anoxic insult.

Proposed Course of the Project: To continue to analyze tissue from asphyxiated neonate, normal animals delivered by Cesarean Sections and normal animals born vaginally.

Publications: None

Serial No. NDB(I) 67-LPP 1465
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH-
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neurometric Studies

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Capt. Douglas B. Tang
Dr. Bruce Konigsmark

Cooperating Units: Walter Reed Army Institute of Research
Johns Hopkins University

Man Years:
Total: .2
Professional: .2
Other: 0

Project Description:

Objectives: 1) To determine the significance of size and numbers of nervous system components. 2) To develop mathematical and histological methods for such quantitative studies.

Methods Employed: During this year a systematic study of several variables encountered in quantitative studies of the central nervous system was continued with the use of the superior cervical sympathetic ganglion as a model. The ganglia were embedded in paraffin, cut serially, and the sections stained for the demonstration of neuronal nucleoli or treated according to the method of Bodian for the demonstration of axons.

Major Findings: The quantitative characterization of highly organized cell populations encountered in the nervous system and the implications for development and function continue to be a major area of interest to the neuroanatomists. Such studies pose many technical and methodological problems not the least of which is the sampling problem. Most quantitative studies have in common the fact that counting or measuring of particles must be carried out in serial histological sections of the cell aggregate under investigation. In but a few instances is it feasible to count or measure the particles in every

section, so that, by necessity, some sort of sampling scheme must be employed. The problem of the selection of sections was discussed in last year's report. This year we have studied the problem of sampling within a given section. The study considers the sampling precision of two sampling procedures for estimating the total number of nerve fibers exposed on cross section of a nerve trunk. Specifically, using the large ocular grid of the microscope to define and locate counting areas (sampling units), the mean square error associated with selecting the sampling units completely at random is compared, in nine populations of nerve fibers, with a version of the frequently employed systematic sampling method of "counting in every Kth sampling unit".

In the nine nerve fiber populations studied, the systematic selection of counting areas was generally superior (at least for small values of K); however, the increase in precision over random sampling was neither dramatic nor predictable. To obtain sampling errors of 10% or less requires, for both procedures, counting of no less than 10% of the available sampling units.

Although the primary emphasis of this study was methodological, with results bases on the study of but one type of nerve fiber populations (preganglionic sympathetic axons), it is felt that certain results will be of practical interest in other similar situations. In addition, it is hoped that this empirical study will motivate further investigations into the sampling errors of the types of sampling methods employed in the quantitative study of the nervous system.

Significance: The need for more quantitative studies of the nervous system is clearly evident to the neuroanatomists, neurophysiologists, neuropathologists, and others. The lack of information in this area is mainly due to the magnitude and tediousness of such studies. Computer analyses and automatic scanning devices are currently being developed for such studies. However, histological and statistical methods must be developed prior to the utilization of such automatic devices.

Proposed Course of the Project: To continue the examination of each variable encountered in quantitative studies of the nervous system and to develop standardized methods for such studies.

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Ebbesson, S. O. E., and Tang, D. B.: A method for estimating the number of cells in histological sections. J. Roy. Microscop. Soc. 84: 449-464, Dec. 1965.

Ebbesson, S. O. E.: Quantitative studies of superior cervical sympathetic ganglia in a variety of primates including man. I. The ratio of preganglionic fibers to ganglionic neurons. J. Morphology.(In press).

Ebbesson, S. O. E.: Quantitative studies of superior cervical sympathetic ganglia in a variety of primates including man. II. Neuronal packing density. J. Morphology (In press).

ARTICLE IN A BOOK:

Ebbesson, S. O. E., and Tang, D. B.: The sampling of structured cell populations. In the Proceedings of the Second International Congress of Stereology. Springer-Verlag, 1967, pp. 131-132.

Serial No. NDB(I) 67-LPP 1466
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electron microscopic study of brain swelling secondary to prolonged partial asphyxia.

Previous Serial Number: Same

Principal Investigators: Dr. Ronald E. Myers
Dr. William Bondareff
Dr. David Brandes

Other Investigators: Dr. A. W. Brann, Jr.
Dr. J. Pysh

Cooperating Units: Northwestern University, The Medical School, Department of Anatomy.

Man Years:
Total: 1.0
Professional: .6
Other: .4

Project Description:

Objectives: Study cellular and brain space (extra and intracellular) changes. Apply electron microscopic histochemical techniques to monkey asphyxia model.

Methods Employed: Fetal monkeys were subjected to periods of prolonged partial asphyxia or to acute total asphyxia. Sections of brain tissue are then taken immediately at termination of procedure and fixed by a number of methods: Osmium tetroxide, freeze dry method, etc.

Major Findings: This project is just in its initial phases and results to date are not sufficient to make definite statements concerning the compartment of brain that increases when the brain swells. There are conflicting results from the material fixed in different ways and this conflict is being explored further. As regards other changes, irregardless of fixation method, the mitochondria are consistently swollen. Histochemical studies suggest increase in acid phosphotase content as lysosomes.

Significance: As yet the significance of the findings can not be stated. This will depend on further study of swollen brains.

Proposed Course of Project: More animals will be done in both the control series and in the experimental series. Various degrees of swelling will be produced in an attempt to give a clearer picture of the progression of the process along with additional calculations of the brain compartments at these various stages of brain swelling. In this way the discrepancies between the intra and extracellular spaces by various fixation methods can be explored further.

Publications:

Brandes, D. and Myers, R. E.: Lysosomes and Anoxic Brain Damage. In James, L. S., Myers, R. E., and Gaull, G. E. (eds.): Brain Damage in the Fetus and Newborn from Hypoxia or Asphyxia. Columbus, Ohio, Ross Laboratories, 1967. pp. 34-36.

Serial No. NDB(I) 67-LPP 1467
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Histochemical alterations following asphyxia.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Igor Klatzo
Dr. Miroslaw Mossakowski
Dr. Helen Rodríguez

Cooperating Units: Laboratory of Neuropathology, NINDB
Institute of Experimental Medicine, Warsaw, Poland
University of Puerto Rico, Department of Pediatrics

Project Discontinued: Completed.

Publications:

Klatzo, I., Mossakowski, M., Myers, R. E., Long, D. M., Rodríguez, H., and Walker, J. T.: Early effects of perinatal asphyxia on the brain. In James, L. S., Myers, R. E., and Gaull, G. E. (eds.): Brain Damage in the Fetus and Newborn from Hypoxia or Asphyxia. Columbus, Ohio, Ross Laboratories, 1967. pp. 17-21.

Serial No. NDB(I) 67-LPP 1471
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cardiovascular and blood chemical responses to acute asphyxia in the monkey fetuses of different gestational ages.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.6 |
| Professional: | 0.3 |
| Other: | 0.3 |

Project Description:

Objectives: To study the cardiovascular and blood chemical effects of acute asphyxia on fetuses of different gestational ages.

Methods Employed: Fetuses of different gestational ages are removed from the uterus still attached to the mother by the umbilical cord. They are placed into a bath of physiological saline maintained at body temperature. The carotid artery of one side is cannulated allowing for continuous recording of blood pressure, heart rate, and for the collection of blood samples at intervals. The fetus then sustains cord compression completely interrupting exchange between mother and fetus for predetermined lengths of time. Fetal pulmonary ventilation is prevented by rubber sacs placed over the head at delivery and prior to first breath. The blood chemical changes and changes in cardiovascular status are recorded. After cord release the fetus may recover spontaneously or it may require resuscitation. After restoration the fetus is returned to the uterus and the hysterotomy opening closed. The fetuses are brought to term and delivered by c-section. Clinical and neuropathological consequences of acute asphyxia are determined.

Major Findings: There are marked differences in response of fetuses of different gestational ages to acute asphyxia as brought about by cord compression. The younger fetus exhibits a marked tolerance to acute asphyxia with a gradual and late loss in blood pressure and a gradual and late decrease in heart rate. Also, blood chemical changes per unit time tend to be less dramatic in the younger fetus. Corresponding with these differences in

response to asphyxia are marked differences in tolerance from the point of view of production of brain damage. The increment in hydrogen ion concentration per unit time is equal throughout the course of asphyxia for each animal whatever gestational age. $pO_2$ drops dramatically from levels of 40mm of mercury at beginning of asphyxia to essentially zero levels by the end of five minutes. The major change occurs during the first two and a half minutes. Throughout the remainder of asphyxia, there is no oxygen availability to the tissues and the animal survives on the basis of anaerobic energy transformations. The total $CO_2$ content of the blood throughout asphyxia remains essentially constant. $pCO_2$ increases from levels of 15 mm of mercury at the beginning to levels approximately 150 to 200 mm of mercury at the end of asphyxia. The increment in partial pressure of $CO_2$, despite the equality of the total $CO_2$ content, relates to the increase in hydrogen ion concentration producing an increment in $pCO_2$. Lactate concentration in the circulating blood at the beginning of asphyxiation is approximately two and a half milligrams per cent and increases during the first ten minutes of asphyxia to levels of approximately 7.5 milligrams per cent and thereafter remains constant. Total buffer base drops from levels of approximately forty to forty-five at the beginning to levels of around twenty-five at the termination of asphyxia so that total buffer base availability has decreased by a factor of two. At the same time, base excess decreases rather dramatically during the course of asphyxia from levels of approximately -2.5 to levels of -20.

Significance: The changes in acid base and in cardiovascular status of the fetus during acute asphyxia are of considerable interest in the study of the pathogenesis of asphyxial brain damage whether in relation to the perinatal period or in the adult. The present series of experiments attempt to determine the normal changes in these parameters during asphyxia in preparation for experimental studies where various parameters may be independently manipulated.

Proposed Course of Project: More cases are required to determine the shapes of the mean curves for sizeable populations of monkey fetuses of different gestational ages in order to have valid comparisons of the changes in relation to gestational ages.

Publications: None.

Serial No. NDB(I) 67-LPP 1472
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Response Patterns of Units in Striate Cortex of the Rhesus monkey

Previous Serial Number: Same

Principal Investigator: Dr. Ronald A. Cyrulnik

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.0 |
| Professional: | .8 |
| Other: | .2 |

Project Description:

Objectives: Locus of response in topographically organized cortex, such as occurs in the visual system, is determined by the special characteristics of the stimulus. The temporal pattern of the response could presumably be the conveyor of information regarding other stimulus characteristics, such as wavelength and intensity. In this project, an attempt is being made to define discharge patterns of units in striate cortex, and the stimulus parameters which determine them.

Methods Employed: Extracellular recording is done in striate cortex of anesthetized Rhesus monkeys whose eyes have been refracted. Using interference filters, visual stimuli of limited area are projected on a screen, and, for a given locus, responses are compared for various wavelengths and intensities.

Major Findings: A unique optical system has been constructed capable of producing an intense 10 millisecond pulse of light with a rise time of less than one millisecond. It utilizes a xenon flashtube, the voltage to which is divided by a 10-section delay line of empirically derived configuration. Artifacts from this have been practically eliminated by shielding and by the construction of an adjustable ground point hum balance which also keeps noise levels extremely low. This arrangement will permit accurate comparisons of response patterns of the units that are being recorded.

Significance: From a fundamental viewpoint, it is of particular interest to determine the extent to which neuronal electrical activity is essential in conveying information. From a practical viewpoint, knowledge of an electrical code which is meaningful to the nervous system presents the possibility of conveying information to the animal directly by such means.

Proposed Course of Project: To continue with project as planned.

Publications: None.

Serial No. NDB(I) 67-LPP 1473
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Effect of Social Deprivation on the Maternal Behavior of Primiparous and Multiparous Female Rhesus Monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Elizabeth A. Missakian

Other Investigator: None

Cooperating Units: None

Man Years:
Total: .10
Professional: .10
Other: .0

Project Description:

Objectives: 1) To investigate the effects of social deprivation on the maternal behavior of laboratory-reared female rhesus monkeys. 2) To study the effect of previous parity on the maternal behavior of laboratory-reared female monkeys.

Methods Employed: Four groups of animals are involved in this study. Group 1 contains primiparous feral-reared animals. Group 2 contains multiparous females who have been reared under free-ranging conditions. Groups 3 and 4 contain laboratory-reared females. Females of Group 3 are primiparous and those of Group 4 are multiparous. (By definition, a laboratory-reared animal is one who has been separated from its mother immediately after birth and raised at the laboratory. It is housed individually and has visual and auditory, but no physical manipulative contact with other monkeys.) In this study, the maternal-infant interaction is observed for two hours daily from parturition until the infant is 50 days of age. Frequency and duration measures of the following behavior categories are recorded: grooming by the mother (one-hand, two-hand, mouth), punishment by the mother (cuff, bite, threat), return signals by the mother (silly grin, affectional present, leg present), maternal

retrieval responses, infant escape responses, general activity of the infant (sleep, activity, locomotion), and general activity of the mother (sleep, activity, locomotion).

Major Findings: Observations have been completed on three multiparous feral-reared, two primiparous feral-reared, three primiparous laboratory-reared females. At present, observations are being made on the behavior of a primiparous laboratory-reared female, thus bringing the total laboratory-reared females observed to four. The major findings to the point are: 1) There is no significant difference in the maternal behavior of multiparous and primiparous feral-reared females; 2) Both multiparous and primiparous feral-reared females groom their infants significantly more than the primiparous laboratory-reared females; 3) There is a higher frequency of infant escapes among infants of feral-reared females; however, the average duration of each escape is longer for infants of laboratory-reared females; 4) There is a significantly higher frequency of maternal return signals executed by feral-reared than laboratory-reared females; and 5) primiparous laboratory reared females are significantly more aggressive toward their infants than are feral-reared animals.

Significance: The findings to date indicate that social deprivation has a detrimental effect on maternal behavior of laboratory-reared primiparous females. The deficit, however, is not so severe as to preclude all maternal responsiveness. Primiparous laboratory-reared females are capable of nursing, retrieving and grooming their infants. Their maternal behavior is not as efficient as that of feral-reared animals. The fact that socially deprived females do care for their infants suggests that the responses necessary for adequate maternal behavior are not learned. Observations have not been made on multiparous laboratory-reared animals. Their behavior patterns will furnish information on the question of whether the effects of social deprivation are irreversible. If the maternal behavior of multiparous laboratory-reared females is normal, it is then reasonable to conclude that deficits observed in primiparous animals are not due to social deprivation per se, but rather to the lack of visual and manipulative contact with infants.

Proposed Course of Project: 1) To investigate the difference in social behavior of infants of feral-reared versus laboratory-reared females following two years of rearing with the respective mothers. 2) To assess the effect of previous

visual and/or physical contact with infants on the maternal behavior of primiparous laboratory-reared females.

Publications: None.

Serial No. NDB(I) 67-LPP 1474
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Effect of Social Isolation on the Reproductive Behavior of Male Rhesus Monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Elizabeth A. Missakian

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | .25 |
| Professional: | .25 |
| Other: | .0 |

Project Description:

Objectives: 1) To determine the extent of the effect of social deprivation on the adult reproductive behavior of male rhesus monkeys. 2) To assess whether the effects of social deprivation are permanent and irreversible. 3) To determine whether the effects of social deprivation are restricted to reproductive behavior or whether they also affect the entire structure of social responsiveness.

Methods Employed: Three groups of adult male rhesus monkeys were observed during the course of this study. Group 1 consisted of feral-reared males. Groups 2 and 3 contained laboratory-reared males. Male monkeys of Group 2 had had social experience prior to the onset of this study. Their experience consisted of occasional matings with sexually receptive females. Males in Group 3 had had no experience with other monkeys prior to the onset of this study. Each male was mated a total of 15 times with sexually receptive females. The initial two hours of the mating were observed; however, the male and female were left together for an additional 22 hours. Frequency and duration measures of the following behavior categories were recorded: grooming by male, grooming by female, aggression (physical) by male, aggression (threat) by male, stereotyped locomotion, self-aggression, masturbation, mount attempt, mount and auto-

manipulation. Twenty-four hours after the initiation of the mating, the male and female were unmated and a vaginal lavage was collected and examined by the investigator for the presence and viability of spermatozoa.

Major Findings: 1) There was a significant difference in the frequency of grooming between feral and laboratory-reared males. The difference between experienced and inexperienced males was not significant. 2) There was a significant difference in the duration of grooming bouts between feral and laboratory-reared males. The difference between experienced and inexperienced laboratory-reared males was not significant. 3) There was a significant difference in the total grooming performed by the female of feral versus laboratory-reared males. The difference between experienced and inexperienced animals was not significant. 4) There was a significant difference in the duration of grooming bouts of females when they were grooming feral versus laboratory-reared males. The difference between experienced and inexperienced monkeys was not significant. 5) Laboratory-reared males were significantly more aggressive than feral-reared males. The difference between experienced and inexperienced males was not statistically significant. 6) Feral-reared males executed significantly more threat responses than laboratory-reared males. The difference between experienced and inexperienced monkeys was not significant. 7) Feral-reared males executed significantly more mount attempts than laboratory-reared males. The difference between experienced and inexperienced males was not significant. 8) Inexperienced laboratory-reared males executed significantly more stereotyped locomotion than experienced laboratory-reared males. 9) There was no difference in the frequency of self-aggression or masturbation between the two groups of laboratory-reared males. 10) A laboratory-reared male was never observed to execute an appropriate mount. 11) None of the vaginal lavages of females mated for 24 hours with a laboratory-reared male contained spermatozoa.

Significance: Perhaps the most significant finding of this study was the complete absence of normal reproductive behavior among male monkeys reared under conditions of social deprivation. Observations of masturbation, auto-manipulation, erection and mount attempt indicated that social deprivation did not act to decrease the sexual drive of laboratory-reared males, but to diminish the appropriate orientation of the drive. The consistent lack of a significant difference between experienced and inexperienced laboratory-reared males indicated that the deficits produced by social deprivation were permanent and could not be reversed by the type of social experience afforded the

experienced group of subjects. Deficits in patterns of behavior such as grooming, aggression, grooming duration suggested that not only reproductive behavior is detrimentally affected by social deprivation, but that the entire structure of social responsiveness is deleteriously affected.

Proposed Course of Project: The specific project investigating the effect of social deprivation of adult reproductive behavior of male monkeys has been completed. A manuscript describing this project has been submitted for publication.

Publications: None.

Serial No. NDB(I) 67-LPP 1475
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Circulatory Stasis and its Neurological Sequellae.

Previous Serial Number: Same

Principal Investigators: Dr. James R. Miller
Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 2.5 |
| Professional: | 1.7 |
| Other: | .8 |

Project Description:

Objectives: To study, experimentally, the patterns of neurological damage, clinically and pathologically, that occur with circulatory stasis. To define the factors which contribute to this damage.

Methods Employed: Monkeys are subjected to total circulatory stasis for timed intervals by clamping the aorta and vena cavae near the heart. This procedure excepts the heart from the insult by maintaining a competent pulmonary and coronary circulation. Following the arrest period, the animals are observed for recovery of neurological function. After an appropriate interval they are sacrificed and neuropathological examinations are performed.

Major Findings: It has been found that the Central Nervous System (CNS) can withstand total ischemia for up to twenty minutes with virtually complete recovery of function. Functional deficits, however, may be found after shorter time intervals. This variability in the time for onset of functional disability and a further variability in degree of deficit at any one time period suggest that factors other than the ischemia itself are playing a role in the production of dysfunction. It has also

been found that animals with severe disability initially may progress to complete recovery. Convalescence may be prolonged and marked improvement has been noted for up to four weeks after the insult.

Pathological examinations to date have confirmed a clinical impression that cerebellum, brainstem, and spinal cord are areas damaged early. Cortical involvement is usually found in relation to profound hypotension and to severe damage elsewhere.

Significance: In clinical medicine the brain is considered to be intolerant to total circulatory arrest with lesions felt to occur within four minutes unless adequate circulation is restored. The present findings suggest that the CNS may be far more tolerant to total than this. Since, in this model, the heart is protected from the insult, it is felt that the early damage seen in human cases may be related to cardiac failure and a compromised cerebral circulation in the post arrest period. The lack of early cortical involvement in this model is in contradistinction to the human situation. Its occurrence here in association with hypotension again suggests the importance of post arrest cardiac compromise in causing deficit. The possibility, therefore, must be considered that, in clinical medicine, improved support of cardiac function may enhance the outcome in cases of circulatory arrest.

Proposed Course of Project: In spite of relative protection of the heart, mortality in these experiments has been high due to a combination of cardiac and respiratory problems. Attempts will be made to improve the procedure and then to delineate the upper limits of brain tolerance to ischemia. Efforts will also be made to determine what other factors may be playing a role in the production of the brain damage.

Publications: None.

Serial No. NDB(I) 67-LPP 1476
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Projections of the Frontal Lobe in the Monkey

Previous Serial Number: Same

Principal Investigators: Dr. James R. Miller
Dr. Ronald E. Myers

Other Investigators: None

Man Years:
Total: .3
Professional: .1
Other: .2

Project Description:

Objectives: To study the projections of the frontal lobe to other brain parts with special emphasis on the intercortical relations.

Methods Employed: A frontal lobectomy was performed and the brain stained utilizing the Nauta technique for axonal degeneration. Serial sections were then examined.

Major Findings: Work to date has established that the frontal lobe projects heavily to the opposite frontal lobe in a strictly homological fashion. Ipsilaterally, the anterior pole of the temporal lobe receives heavy projections. The rest of the cortex appears to be virtually without projections from the frontal lobe.

Significance: The development of the silver staining techmiques for axonal degeneration allows for a more comprehensive and accurate description of cortical projections than has been possible in the past. Particularly lacking are adequate studies of intercortical connections. It is expected that these anatomical studies will complement and clarify the evidence of neuronal connectionism being obtained by neurophysiological techniques.

Proposed Course of Project: Demands of other projects on the professional staff's time have precluded work on this

problem in the present year. Work is to continue with total lobectomies to complete the description of cortical and subcortical projections. This will be followed at a later date by more specific lesions of particular parts of the frontal lobe.

Publications: None.

Serial No. NDB(I) 67-LPP 1477
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Neural Control of Facial Expression in the Monkey

Previous Serial Number: Same

Principal Investigators: Dr. James R. Miller
Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .3
Professional: .3
Other: 0

Project Description:

Objectives: To study brain areas involved in facial movements.

Methods Employed: The effects of lesions of cortical and sub-cortical centers on facial movements are being examined. Spontaneous and emotionally elicited movements are observed as the important indices of deficit.

Major Findings: Extensive lesions of cortex produce only slight to moderate amounts of facial weakness. Even total ablation of the motor cortex does not cause profound loss of spontaneous movements or any loss of response to strong emotional situations. Deficits similar in degree to that found with lesions of the motor cortex, have also been found with ablations of the pre-frontal area and of the temporal lobe.

Significance: In humans, dissociation between volitional and emotional control of facial expression has long been noted. Although volitional activity has been related to the facial area of the motor cortex, areas which contribute to the emotionally generated movements are poorly known. In the monkey it appears, from the present work, that the motor cortex is far less prominent in its contribution to facial movements than in the human.

The monkey utilizes facial movements, primarily, if not exclusively, in emotional responses. While cortical areas studied to date seem to have little effect upon these responses, it is expected that this investigation will provide information on the areas that do contribute to emotional facial expression.

Proposed Course of Project: Demands of other projects on the Professional Staff's time have precluded work on this project in the present year. Examination of the effects of cortical lesions is to be completed and attention will then be turned to sub-cortical areas.

Publications: None.

Serial No. NDB(I) 67-LPP 1478
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Neural Control of Hand Strength in the Monkey

Previous Serial Number: Same

Principal Investigators: Dr. James R. Miller
Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .6
Professional: .6
Other: 0

Project Description:

Objectives: To define, in a precise manner, the contribution of particular brain areas to motor function, as tested by the parameter of hand strength.

Methods Employed: A machine is utilized which tests a monkey's strength by having him squeeze two levers together to obtain food reward. The amount of force required can be varied by known amounts. When a monkey's maximum capability has been determined, neurosurgical procedures are performed and their effects on hand strength evaluated.

Major Findings: Preliminary investigations with a device which did not totally exclude the use of proximal arm muscles indicate that extensive lesions in the hand motor cortex have to be made before any deficit in tests of arm and hand strength becomes apparent. Smaller lesions produced no effect on strength no matter where their location in the hand area. When effects were seen on strength there tended to be virtually total loss of power in the extremity.

Significance: Little is known about the contributions of the major parts of the motor mechanism to motor performance and motor strength. The present study with lesions of the motor

cortex attempt to define clearly the effects of this important brain area on these parameters.

Proposed Course of Project: Demands of other projects on the professional staff's time have precluded work on the project in the present year. At a latter date evaluation of the effects of motor cortex and other cortical areas on these parameters will be completed.

Publications: None.

Serial No. NDB(I) 67-LPP 1479
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** The effect of visual information on intermanual transfer of problem solving in the monkey.

**Previous Serial Number:** Same

**Principal Investigator:** Dr. Bruno Kohn

**Other Investigators:** Dr. Ronald E. Myers

**Man Years:**

| | |
|---|---|
| Total: | .5 |
| Professional: | .5 |
| Other: | -- |

**Project Description:**

Objectives: To determine the effect of visual input on the intermanual transfer of a problem solving task in commissure-sectioned monkeys.

Methods Employed: Monkeys with anterior commissure and corpus callosum sectioned and unoperated control animals were tested for intermanual transfer of latch-box problem solving. Two different conditions were used in training and test:

Visual condition: Animals could view their arm and the problem box freely while working.

Non-visual condition: Animals could view the problem box only when not working on it. During the manipulation of the box the animals were prevented from seeing either their arm or the box itself.

Five groups of animals were run in the following manner:

| | Training | Test |
|---|---|---|
| Normal group | No vision | No vision |
| Operated group | No vision. | No vision |
| " " | Vision | Vision |
| " " | No vision | Vision |
| " " | Vision | No vision |

Major Findings: The normal group showed intermanual transfer. All operated animals trained in the no vision condition required retraining with the second arm. Operated animals trained with vision were able to solve the problem on the test when vision was allowed, whereby half of the animals in the last group performed well in the no vision test condition.

Significance: The data confirm the involvement of forebrain commissures in the intermanual transfer of a motor skill. Moreover, they show that in absence of normal commissural function on visual stimulation during training can maintain the transfer of problem solving.

Not only can visual memory mediate the test performance, but it can also sustain this skill without further visual input. These results suggest that a somatomotor response pattern can be encoded in visual modality and reproduced largely on basis of prior visual input.

Proposed Course of Project: In the second part of this study, animals with optic chiasma section as well as commissurotomy will be used. In training and testing the animals monocularly in ipsilateral and contralateral eye-hand combinations it is expected to gain information about the following questions:

1. Relative contributions of visual and somatomotor input to problem solving at different stages of training,

2. Attentional processes in the two modalities,

3. Capacity for interhemispheric sensori-motor integration.

Publications: None

Serial No. NDB (I) 68 1556
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The effects of cerebral lesions on the social behavior of free-ranging Rhesus monkeys.

Previous Serial Number: None

Principal Investigators: Dr. Chester P. Swett, Jr.
Dr. Ronald E. Myers

Other Investigators: None

Cooperation Units: None

Man Years:
Total: .4
Professional: .4
Other: .0

Project Description:

Objectives: To study the effects of brain lesions on the social behavior of free-ranging Rhesus monkeys.

Methods Employed: Preoperative observations were carried out using members of Group E at Cayo Santiago, Puerto Rico. Data on social groupings, grooming, and aggression were summarized. Postoperative observations are in progress.

Nine animals have been trapped and operated to date:

| | |
|---|---|
| Bilateral anterior temporal lobectomies | 5 |
| Bilateral prefrontal lobectomies | 2 |
| Bilateral partial anterior temporal lobectomies | 2 |
| Pinealectomies | 2 |

Major Findings: Animals with total anterior temporal lesions do not return to their group. They lead a solitary existence. 2. Animals with prefrontal lesions also lead a solitary existence and fail to rejoin their social group. 3. Animals with partial anterior temporal lobectomies rejoin their group and do not exhibit abnormal behavior patterns. 4. Pinealectomized animals return to their social group and appear to engage in normal behavior patterns.

Significance: It is well known that caged monkeys exhibit behavior patterns

quite different from animals in the wild. The free-ranging monkeys of Cayo Santiago provide an opportunity of examining the social behavior of animals with brain lesions in a naturalistic environment. The results indicate that both the temporal and the prefrontal lobus function prominently in the definition of social behavior.

Proposed Course of the Project: 1. To determine the behavioral basis for the assumption of solitary existence by the lesioned animals. 2. To determine the portions of anterior temporal cortex important to social behavior.

Publications: None

Serial No. NDB (I) 68-1557
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Daytime sleep patterns in a band of free-ranging Rhesus monkeys

Previous Serial Number: None

Principal Investigator: Dr. Chester P. Swett, Jr.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .6
Professional: .6
Other: .0

Project Description:

Objectives: 1. To investigate the amount of daytime sleep which occurs in Rhesus monkeys of both sexes and various ages. 2. To determine if rapid-eye-movement (REM) sleep occurs during the day.

Methods Employed: One animal of each sex from ages 1-6 was studied as this range spans the developmental period of a Rhesus monkey's life. In addition, two adults (6+ years) of each sex were studied. The behavior of each monkey was studied for 20 minutes out of each hour from 8:00 a.m. to 4:00 p.m. Sleep intervals, position and location were recorded.

Major Findings: During the day Rhesus monkeys sleep mainly from 10:00 a.m. to 3:00 p.m. when the animals gather in small groups. Females sleep 11.3% in an hour, but sleep occurs over 20% of the time from 10:00 a.m. - 1:00 p.m. Males sleep 2.4% in an hour or significantly less ($p > .05$) than females.

Females sleep sitting on the ground in nearly 2/3 of the cases. In contrast, males nearly always sleep on the ground and sit or lie in equal ration.

Rapid-eye-movement (REM) sleep occurs rarely during the day. However, the mean duration of sleep intervals in which REM sleep occurred was five intervals longer than sleep intervals with no REM's.

Significance: Sleep is one of the most common activities of mammals. Yet it has been studied very little in free-ranging populations. The results of this study show that sleep occurs during the daytime and can be quantified

even in naturalistic conditions. It is now evident that Rhesus monkeys change their sleep patterns according to their new environment. Characterization of sleep and rest patterns is of value in attempting to understand sleep as a form of behavior which may be modified somewhat to suit the needs of the organism.

Proposed Course of the Project: Further studies of rest locations and associations of animals while resting are in progress.

Publications: None

Serial No. NDB (I) 68-1558
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Primate social patterns

Previous Serial Number: None

Principal Investigator: Dr. Donald Stone Sade

Other Investigator: Mr. James D. Loy

Cooperating Units: Northwestern University, Evanston, Illionis

Man Years:

| | |
|---|---|
| Total: | 2.5 |
| Professional: | 2.5 |
| Other: | 0.0 |

Project Description:

Objectives: To identify and describe the mutual constraints of interacting life cycles of Rhesus monkeys (Macaca mulatta) maturing within a social group which has itself developed with minimum disturbance under free-ranging conditions.

Methods Employed: Daily records on behavior of all individuals within one social group (Cayo Santiago Group F) are analyzed using ethological and sociometric techniques. Observations on the same individuals have been carried out by the principal investigator since 1960. Life histories are constructed from the longitudinal records so obtained.

Major Findings: The major predictive variable in Rhesus social organization is genealogical. Dependant variables include dominance status within age classes and between genealogies. Adult reproductive behavior, departure of adolescent males from the natal group, companionships of adolescent males following departure from natal grou, and various subsidiary problems are under investigation.

Proposed Course of the Project: The number of known individual life histories will be increased by continued and continuous observation of Cayo Santiago Group F.

Serial No. NDB (I) 68-1558

Publications:

Sade, D.S.: Determinants of dominance among free-ranging Rhesus monkeys. In Altman, S. A. (Ed.): Social Communication Among Primates. University of Chicago Press, 1967, pp. 99-114.

Sade, D.S.: Inhibition of son-mother mating among free-ranging Rhesus monkeys. Science and Psychoanalysis, 1968, (in press).

Serial No. NDB (I) 68-1559
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The effect of amygdalectomy on social behavior of free-ranging Rhesus monkeys.

Previous Serial Number: None

Principal Investigators: Dr. Arthur Kling, Dennis Dicks

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: Department of Psychiatry, University of Illinois at the Medical Center.

Man Years:
Total: .6
Professional: .4
Other: .2

Project Description:

Objectives: The effects of removal of the amygdaloid nuclei upon the social behavior of Rhesus monkeys are being studied.

Methods Employed: On Cayo Santiago observations over 8 to 12 weeks before operation will provide adequate with-in subject controls; observations of the operated animals will continue for the same period in order to control for seasonal change in behavior.

In the field the observer will attend to five types of interactions: sex, grooming, aggression, grouping and feeding. The communicative signals leading to and accompanying such interaction will be of particular interest, since there is some evidence that, in a social context, amygdalectomised monkeys ignore normally effective comminication (Kling, Dicks and Gurowitz (forthcoming).

Significance: This study will be one of the first to examine operated animals functioning in their normal environment. It may elucidate the role, if any, of the amygdaloid complex in delicate social processes.

Proposed Course of the Project: At the moment, preoperative observations are underway. Depending upon the results, it is hoped that more refined studies will be feasible either in the field at Cayo Santiago, or in the "enclosures" projected by LPP, San Juan.

Serial No. NDB (I) 68-1559

Publications: None

Serial No. NDB(I) 68-LPP 1560
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effects of prolonged partial asphyxia on the fetal and neonatal nervous system of the monkey.

Previous Serial Number: NDB(I) 67-LPP 1468; NDB(I) 67-LPP 1469, and NDB(I) 67-LPP 1470.

Principal Investigator: Dr. A.W. Brann, Jr.

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total:
Professional:
Other:

Project Description:

Objectives:

1) Outline the perimeters under which brain swelling occurs in prolonged asphyxia.

2) Compare and contrast brain pathology seen in prolonged partial asphyxia versus that seen in acute total asphyxia.

Methods Employed: Term fetal monkeys with catherization of fetal vessels and electrocardiographic leads were subjected in utero to a period of hypoxia with its associated metabolic and respiratory acidosis.

Major Findings: When the fetal status has deteriorated such that blood pH is below approximately 7.00 for as long as 2 hours, the brain is swollen at delivery. With failure to survive beyond the first day of life, examination of the brain revealed, in addition to brain swelling: a) focal areas of hemorrhagic cortical necrosis frequently involving the paracentral regions symmetrically; b) generalized cortical necrosis with or without a hemorrhagic component. The presence in these cases of brain swelling and hemorrhagic cortical necrosis after prolonged partial oxygen deprivation is in marked contrast to the absence of brain swelling and the prominence of brainstem lesions after acute total asphyxia. In the monkeys

who survived the initial insult for 4-6 months, atrophic cortical sclerosis has been found along with destructive changes in the basal ganglia.

Significance: The findings of atrophic cortical sclerosis in animals who have had prolonged partial asphyxia gives insight into the pathogenesis of the identical counter part of this brain lesion in man.

Proposed Course of Project: More fetuses will be compromised to outline the minimal insult necessary to produce damage to the fetus. Physiologic data will be collected in attempt to outline more clearly how fetal compromise occurs in this maternal-fetal preparation.

Publications: No.

Serial No. NDB(I) 68-LPP 1561
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemistry of the Recovering Juvenile from Circulatory Stasis.

Previous Serial Number: None

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. James R. Miller

Cooperating Units: None

Man Years:
Total: 1.0
Professional: .4
Other: .6

Project Description:

Objectives: To study the biochemical changes in the brain and other tissues of the juvenile recovering from circulatory stasis.

Methods Employed: Monkeys are subjected to 14 minutes of peripheral circulatory arrest by clamping the great vessels which enter and leave the heart (Project No. 67-LPP-1475 by Dr. James R. Miller). The animal is allowed to recover and sacrificed at various times after the insult. The brain, liver, spleen, kidney and muscle are quickly excised from the anesthetized monkey and frozen in a mixture of isobutane and isopentane colled to the temperature of liquid nitrogen.

Major Findings: The data is not complete but certain facts are available. The brain of the juvenile contains 0.5 mg of glycogen per gram wet weight. This value is similar to that found in the monkey neonate. The liver contains only 43 mg per gram wet weight, approximately 40% that found in the neonate. The glycogen levels in the brain are depleted by 60% during the circulatory arrest, but recover rapidly. Twelve hours after the insult it is 400% above control values. Sham operations show that these dramatic changes in brain glycogen are due to the trauma of the circulatory arrest and not due to the trauma of surgery.

Significance: It is expected that this study will give us an insight and understanding of the biochemical problems affecting the

survival of these animals subjected to circulatory stasis.

Proposed Course of Project: Complete the assay of the other organs (kidneys, spleen and muscle) and try to correlate this experiment with those of acute asphyxia.

Publications: No.

Serial No. NDB(I) 68-LPP 1562
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemistry of the recovering chronically asphyxiated monkey brain.

Previous Serial Number: None

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. A.W. Brann, Jr.

Cooperating Units: None

Man Years:
Total: 0.1
Professional: 0.1
Other: 0

Project Description:

Objectives: It is proposed to study the levels of various substances in the brain and other tissues of the monkey neonate recovering from chronic asphyxia.

Methods Employed: The monkey term baby is to be delivered and manipulated as outlined by Dr. A.W. Brann, Jr., in Project No. 68-1560. The monkey babies are to be sacrificed at various time intervals after the insult. Normal babies delivered by Ceasarean Section are to be used as controls. The brain, heart, lungs, liver, spleen, kidney, muscle and blood are to be rapidly removed from the anesthetized monkey, and quickly frozen (at the temperature of liquid nitrogen) in a mixture of isobutane: isopentane. The tissues are to be stored at -20°C.

Major Findings: This project is still under development.

Significance: To learn the biochemical processes involved in the recovery of the brain and other organs from chronic asphyxia and to determine what chemical changes are associated with brain damage and recovery from damage.

Proposed Course of Project: To continue to establish the technique and methods necessary for this particular project and to study the glycogen levels of the recovering chronically asphyxiated brain.

Publications: No.

Serial No. NDB(I) 68-LPP 1563
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemistry of in-vivo brain swelling in the monkey.

Previous Serial Number: None

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. Harvey Shapiro

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0 |

Project Description:

Objectives: It is proposed to study the level of various substances in the brain and other organs of the monkey juvenile during experimentally induced in vivo brain swelling.

Methods Employed: The monkey juvenile shall be subjected to an hypoxic episode while under anesthesia as outlined by Dr. Harvey Shapiro in Project Number 68-1565. The subject will be sacrificed at the height of its brain swelling syndrome. The brain and other organs shall be removed and frozen or processed, depending on the substances to be assayed.

Major Findings: This project is still under development.

Significance: To learn the biochemical processes involved in vivo brain swelling and to determine what chemical changes are associated with brain damage.

Proposed Course of Project: To continue to establish the techniques and methods necessary for this particular project.

Publications: No.

Serial No. NDB(I) 68-LPP 1564
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Metabolic Factors Involved in the Production of Hypoxic Cerebral Edema

Previous Serial Number: None

Principal Investigator: Dr. Harvey M. Shapiro

Other Investigators: Dr. Americo Rivera, Jr.
Dr. Ronald E. Myers

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 1.0 |
| Other: | 1.0 |

Project Description:

Objectives: Determination of the metabolic factors resulting in cerebral edema in the monkey during an hypoxic insult. Continuous monitoring of the changes in intracranial pressure and in electrical impedance of the brain during the insult and course of developing edema and its resolution when this occurs. Correlation of these parameters with changes in certain aspects of the biochemistry of the brain as outlined by Dr. Americo Rivera, Jr. in Project No. 68-1563.

Methods Employed: Under intravenous barbiturate anesthesia (or Halothane-nitrous-oxide) the adult monkey is exposed to a nitrogen-oxygen gas mixture sufficient to produce an arterial $pO_2$ in the range of 17 - 23 for periods of time varying from 30 minutes to 150 minutes. The animal's respiratory rate and volume are adjusted to yield a normocapnic state during a control period and this mechanical ventilation is maintained through the period of hypoxic insult on into the recovery phase when the animal is able to regain its own respiratory control satisfactorily. The systemic arterial pressure, venous pressure, and EKG are continuously monitored. The blood pH, $pO_2$, $pCO_2$, and base excess are measured every 5-10 minutes and the EEG occasionally monitored. Depending upon the course of the insult the animals divide themselves into two groups. Group 1 contains those that develop profound cerebral edema within 3-5 hours after the hypoxic period. Group 2, after a variable period of weakness and lethargy, go into a phase of recovery with or without subsequent neurologic deficit.

These animals are continuously monitored at less frequent intervals as outlined above. Their brains are later examined from a neurohistopathological standpoint.

Major Findings: This study has developed a model for the production of cerebral edema which excludes the traditional mechanical traumatic edemas, edemas induced by a toxin, and edemas produced through alterations in systemic blood pressure or osmolarity. It appears that severe hypoxia in the absence of severe acidosis does not produce profound cerebral swelling.

Significance: The production of cerebral edema by a graded hypoxic insult will permit further elucidation of the respective roles of hypoxia, acid-base disequilibrium (respiratory and metabolic) systemic blood pressure and various other physiologic parameters in the production of a traumatic cerebral edemas.

Proposed Course of Project: With the stabilization of the experimental model studies will be performed on the biochemistry of the hypoxic edema. Brain water content will be measured as well as brain and blood electrolyte content. Other biochemical studies are under development as noted in Dr. Americo Rivera's Project Report No. 68-1563. In order to allow a study of the process of developing edema brain electrical impedance changes and changes in intracranial pressure will be monitored and sacrifice of the animals timed according to these parameters.

Publications: No.

Serial No. NDB (I) 68-1565
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Prolonged hypoxia and symmetrical white matter necrosis in adult monkey.

Previous Serial Number: None

Principal Investigator: Dr. Harvey M. Shapiro

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 1.0 |
| Other: | 1.0 |

Project Description:

Objectives: To determine the parameters of an hypoxic insult that has produced severe symmetrical white matter necrosis with involvement of one or both divisions of the globus pallidus bilaterally. This project is an outgrowth of Project No. 68-1565 which is concerned with the metabolic factors involved in the production of a traumatic hypoxic cerebral edema.

Methods Employed: The adult monkey, under either light intravenous barbiturate or Halothane-nitrous-oxide anesthesia is exposed to a nitrogen-oxygen gas mixture that results in arterial oxygen tension varying between 17 and 23. This insult is begun after a suitable control period of mechanical volume regulated respiration, which is then maintained at the control constant for the remainder of the experimental insult. The duration of this insult is varied and the animal's mean blood pressure is maintained above a mean value of 50 mm. Hg. The pH, $pCO_2$ $\bar{p}O_2$, and base excess are monitored frequently. Continuous recordings of the systemic arterial and venous pressures and the electrocardiogram are taken. The EEG is occasionally monitored. These measurements are continued throughout control, insult, and recovery periods. Those animals surviving three or more days are examined neurologically and a gross and neuropathological study of the brain and spinal cord are performed.

Major Findings: The production of symmetrical white matter and basal ganglia lesions is a rare occasion in the monkey. It appears that the duration of the metabolic acidosis induced by the hypoxia is a prime factor in the production of the above pathology. Treatment with large doses of

bicarbonate concomitant to the hypoxic insult may preclude the development of the lesions.

Significance: These studies have established that a model for symmetrical lesions in the white matter and basal ganglia is feasible. This will allow further exploration of a lesion complex in the primate that is similar to that seen in humans.

Proposed Course of the Project: Additional animals will be studied under the above conditions to more clearly define the roles of hypoxia, acid-base disequilibrium, and systemic blood pressure in the production of this unique lesion.

Publications: None

Serial No. NDB(I) 68-LPP 1566
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Functions of the anterior corpus callosum in the monkey

Previous Serial Number: None

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | .9 |
| Professional: | .4 |
| Other | .5 |

Project Description:

Objectives: To determine what functions are transmitted inter-hemispherically through the anterior portion of the corpus callosum in the monkey.

Methods Employed: Five experimental monkeys underwent midline section of the corpus callosum in its entire antero-posterior extent, the anterior commissure, and the optic chiasm. Three other monkeys received midline section of the optic chiasm through a small cut made in the middle of the corpus callosum.

Two weeks after surgery, all monkeys received training on delayed response, go-no-go visual color discrimination and delayed alternation tasks with one eye occluded and the arm contralateral to the occluded eye restrained. Initial training proceeded with the same eye and arm till a criterion was met. Transfer of the training to the opposite eye and arm combination was tested after initial training on each task was completed.

Major Findings: It was found that the transfer of the go-no-go task was lacking in the experimental monkeys but was present perfectly in the controls. However, the transfer of delayed response training was equivocal. On the first day of delayed response transfer test, the performance levels were significantly lower than the criterion level

achieved by the trained side, but on the second day, the levels were almost as high as the criterion and remained so throughout the test period.

Training of delayed alternation is complete in a few of the animals, but they suggest that there is good transfer of the training. The performance of the control animals in delayed response and delayed alternation indicated perfect transfer of these training took place.

Significance: The project is an attempt to obtain more information regarding functions of the anterior corpus callosum in the monkey.

The above results raised many questions. Available anatomical and behavioral evidence led to the expectation that monkeys with the midline-sectioned forebrain commissure show lack of transfer of training on delayed response and delayed alternation, and to the expectation that the transfer of go-no-go training would be poor even in the animals with the anterior corpus callosum intact. It has been shown that delayed response and delayed alternation learning depend on the dorsolateral portion of the prefrontal cortex, whereas the ventral portion of the prefrontal cortex is more strongly associated with the learning of go-no-go task. Anatomical evidence indicates that bilateral interconnection between two cerebral hemispheres in the dorsolateral prefrontal area is substantial, whereas the fiber connections between the two sides in the ventral prefrontal cortex appears poor.

Proposed Course of the Project: To continue as planned, and add more experiments to further illuminate on the problems raised.

Publications: None.

Serial No. NDB(I) 68-LPP 1567
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Social organization of a large group of free ranging monkeys as reflected in their grooming.

**Previous Serial Number:** None

**Principal Investigator:** Dr. Margaret Varley

**Other Investigators:** None

**Cooperating Units:** None

**Man Years:**

| | |
|---|---|
| Total: | .5 |
| Professional: | .5 |
| Other: | 0 |

**Project Description:**

Objectives: To describe the social structure of a large group (250 monkeys) of free-ranging Rhesus monkeys on Cayo Santiago, as revealed in their choice of grooming partners. This structure will be compared with those of other groups on the island, particularly another large band has been described by observers as tighter and less diffuse in its organization. When possible, to explore the relation of this structure to behaviors such as dominance, consortship and adult male-young behavior, and to use this information in the study of the other large group on the island.

Methods Employed: Field observation of the monkeys of this group. An attempt is made to identify the monkeys involved in every instance of grooming which is seen. The family relations of each monkey born into the group has been plotted, and the data are analyzed with respect to whether the grooming partners belong to the same (familial) or different (cross-geneological) families. Because grooming reflects some degree of association or intimacy between monkeys, picture can be drawn from the cross-geneological data of direct connections and linkages among the families of this group.

Further analysis of the data will be made in terms of age, sex, and kind of family relationship.

Major Findings: In three months of observation in 1967, 1295 instances of grooming were recorded, of which 125 yielded cross-geneological informa-

tion. This sample must be enlarged considerably by observations in 1968. Tentatively it may be said that (1) the percentage of familial out of total grooming is 70, a proportion roughly equivalent to that of a much smaller band on the island and (2) the group seems to be divided into two smaller groups of roughly equal size. These two groups in turn may be thought of as being connected by four families who "bridge" these groups. More data may show either that a circular arrangement of families is the simplest structure which can be drawn or that a more diffuse set of connections exists across all families.

Significance: The size of rhesus groups on Cayo Santiago and in the temples of India has been reported as usually falling between 27 and 70 members. This raises the question of how the members of a very large band arrange themselves, how cohesive a group they form, and whether they divide themselves into functionally smaller groups.

Proposed Course of the Project: Study of this group will be terminated with its removal from the island, scheduled for the winter of 1968-69. Information gathered with this group will be used in the study of another large island group, currently being investigated by another observer.

Publications: No.

Serial No. NDB(I) 68-LPP 1568
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Development of aggressive response in a Rhesus monkey.

Previous Serial Number: None

Principal Investigator: Dr. Margaret Varley

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .5
Professional: .5
Other: 0

Project Description:

Objectives: To study the development of social behavior in the rhesus monkey, with particular attention to aggressive, submissive and sexual behavior. To specify the emergence of this behavior in the infant with reference to interactions with adults versus peers.

Methods Employed: Observation of the behavior of two groups of monkeys taken from Cayo Santiago and placed in gang cages (8 by 20 feet). One group of monkeys is made up of three adult females who are sisters and should yield three infants. The other group contains two adult females (an aunt and niece) and one male (son of the older female) and should yield one infant. Behavior to be recorded includes those commonly employed to describe individual monkey activity and interactions. The point of reference will be the activity of the infant, with specification of as much context as possible. The behavior of the three infants in the first gang cage will be analyzed with respect to those interactions which are directed toward one another (peers) and those directed toward adults. These infants will be compared with the infant in the second gang cage, who will be reared only with adults.

Major Findings: Because of birth season on the island, this study is in its beginning phase. At this time there has been one birth; two more deliveries are expected. There is reason to believe that the female in the second cage who was pregnant when examined January 29th is now carrying a dead fetus. If she has not produced a live baby within two weeks, an attempt will be made to induce her to adopt a newborn from the laboratory.

Serial No. NDB(I) 68-LPP1568

Significance: A detailed description of the development of aggressive and submissive behavior from its early appearance to its full adult form, and the contexts in which it emerges, is lacking in the literature. This description is needed for an understanding of the behaviors themselves and of the developing infant. The study should also help to clarify the relative importance of experience with peers and adults, a problem raised by the deprivation experiments of Harlow's laboratory.

Proposed Course of the Project: If possible these infants will be followed into their second year of life. The information obtained from the study may be used to plan studies of the development of dominance within and across two groups of infants of mothers who are known to differ in their power relations, ideally two families with clearly different statuses within their group on Cayo Santiago.

Publications: No.

Serial No. NDB(I) 68-LPP 1569
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The neuroanatomical basis of reproductive activity in male Rhesus monkeys.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers
Dr. Elizabeth A. Missakian

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | .10 |
| Professional: | .10 |
| Other: | 0 |

Project Description:

Objectives: To determine the effect of specific neuroanatomical lesions on the reproductive behavior of male Rhesus monkeys.

Methods Employed: Pre-operative measures of the reproductive behavior of adult feral-reared male monkeys are recorded. Each male is mated a total of ten times, the initial two hours of each exposure being intensively observed. The following behavioral measures are recorded: male grooming, female grooming, male grooming duration, female grooming duration, male aggression, male threat, mount, masturbation, and mount attempt. Following the completion of the pre-operative exposures, brain lesions are made and sufficient recovery time is allotted prior to the initiation of the post-operative mating exposures. Ten post-operative matings are observed for each male monkey. The exact same measures are employed to record the post-operative exposures as were used for the pre-operative matings.

Major Findings: At this time, two males have been lesioned and their post-operative mating performance is being observed. The following two lesions have been made: bilateral removal of the amygdala and bilateral removal of the temporal poles. A comparison of pre- and post-operative scores on measures of male grooming, female grooming, male grooming duration, female grooming duration, male aggression, male threat, mount, masturbation and mount attempt indicates no difference in the pre and post-

operative behavior of either of these two males. The post-operative behavior of each male is well within the range of pre-operative behavior.

Significance: To further the understanding of the neuroanatomical basis of reproductive behavior in infra-human primates.

Proposed Course of Project: 1) Observations will continue to be made of the reproductive behavior of the experimental males described above in order to determine whether there is a gradual, long-term effect of either lesion on mounting activity. 2) Additional male monkeys will be lesioned in order to determine the effects of different lesions on the reproductive behavior of such animals.

Publications: No.

Serial No. NDB(I) 68-LPP 1570
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The effect of prolonged social experience on the reproductive behavior of laboratory-reared male Rhesus monkeys.

Previous Serial Number: None

Principal Investigator: Dr. Elizabeth A. Missakian

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total | .15 |
| Professional: | .15 |
| Other: | 0 |

Project Description:

Objectives: To determine the effect of prolonged social experience on the reproductive behavior of adult male laboratory-reared Rhesus monkeys.

Methods Employed: Adult male laboratory-reared male monkeys, both with and without prior social experience, are housed for six months in 10' x 20' gang cages with five additional monkeys. Each of these five animals is feral-reared. Two of these monkeys are sub-adult males and the remaining three are adult females. The social behavior of these monkeys is observed for 30 minutes each morning. The following behavior categories are employed to record the activities of the six animals: male grooming, female grooming, male grooming duration, female grooming duration, male aggression, male threat, female aggression, female threat, masturbation, erection, auto-manipulation, homosexual mounting, heterosexual mounting, mount attempt. Following the six month period of exposure in the gang cages, each male is returned to his home cage. At that time, weekly exposures to sexually receptive females are initiated. Each exposure is of 24 hour duration and the following measures are recorded: male and female grooming, male and female aggression, stereotyped locomotion, self-aggression, masturbation, mount attempt, mount. The pre and post-gang cage exposure behavior of each laboratory-reared male will be compared.

Major Findings: Two male laboratory-reared monkeys, one with prior social experience and one without prior experience are presently in two

separate gang cages with five animals each. At this time, it is not possible to make any definitive statements regarding the social behavior of either of these males.

Significance: This study is important in understanding 1) the type of experience necessary in the development of normal patterns of reproductive behavior and 2) the reversibility of the effects of social deprivation.

Proposed Course of the Project: An additional six males will be introduced into social situations and social groups in the gang cages. If data on the total of eight subjects fails to reveal significant improvements in the social behavior of laboratory-reared males given prolonged social experience, then an additional group of animals will be incorporated into the study. This group will be exposed to a social group for a more extended period of time.

Publications: No.

Serial No. NDB(I) 68-LPP 1571
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Internal Social Organization in a Large Group of Free-Ranging Rhesus Monkeys

Previous Serial Number: None

Principal Investigator: Dr. Elizabeth A. Missakian

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .30
Professional: .30
Other: 0

Project Description:

Objectives: To study the internal group organization, as reflected by grooming relationships, of a large group of free-ranging rhesus monkeys on Cayo Santiago. In addition, to compare the social organization of this group to that of Group C, a large band of monkeys presently under study by a different investigator.

Methods Employed: Observations of the behavior of free-ranging monkeys of Group A on Cayo Santiago are carried out for eight hours per day, two days per week. Special emphasis is placed on recording instances of grooming between members of this social group. Data is analyzed according to the following characteristics of the monkeys: age, sex, familial association, parity status, familial relationship (aunt, niece, grandmother, etc.). Instances of non-familial or cross-geneology grooming reflect positive bonds between members of the group. This measure is the primary indicant of group cohesion and organization.

Major Findings: This study is now in progress.

Significance: Previous studies of internal organization of bands of free-ranging primates have been conducted with relatively small groups of monkeys. Data collected on Group A, which contains approximately 200 members, will provide a basis of comparison with small groups. In

addition, comparisons will be made of the organization of this group with that of Group C. Data on the grooming relationships among animals in Group C are presently being collected by an additional investigator. Attempts will be made to co-ordinate the findings of these two studies in an attempt to understand the dynamics of group organization and cohesion.

Proposed Course of the Project: Observations of the behavior of Group A animals will be continued throughout the following two years. Short and long-term changes in the grooming relationships and internal group organization during breeding and birth seasons will be studied. In addition to data collection pertinent to group organization, future research will focus on the problems of maternal behavior, reproductive behavior and dominance characteristics of adult male monkeys.

Publications: None

Serial No. NDB(I) 68-LPP 1572
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Role of Tectal Commissures in Inter-ocular Transfer of Learning in the Pigeon

Previous Serial Number: None

Principal Investigator: Dr. Laurence J. Stettner

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total: .5
Professional: .5
Other: 0

Project Description:

Objectives: To determine the locus of transfer of visual information from one side to the other in the avian brain.

Methods Employed: Pigeons are trained to make a visual discrimination between a vertical and horizontal line. Prior to training, metal goggles have been cemented to the skull of all pigeons, so that occluders can be placed over either eye. Learning takes place with one eye, and after the pigeon has learned to peck vigorously at one of the stimuli and to refrain from pecking at the other one, he is tested with the untrained eye. During testing, no reward is present so that any tendency for the bird to respond differentially to the stimuli must be a carry over from previous learning. Three subjects are normal, unoperated controls. Five had posterior and tectal commissures sectioned prior to training.

Major Findings: Normal birds show excellent inter-ocular transfer when trained in this way. Operated birds have not yet reached the final testing phase, but pre-training data strongly suggest that at least three of them will show a severe deficit in transfer.

Significance: Birds have a high degree of learning capacity despite their very primitive cortex. This makes their brain particularly interesting for research on the neurological basis of learning. They have no corpus callosum, and the structures involved in inter-ocular

transfer have not been identified. Once they are identified, split-brain bird preparations could be an exceedingly valuable tool in probing the functional organization of the avian brain.

Proposed Course of the Project: To continue testing as planned.

Publications: None

Serial No. NDB(I) 68-LPP 1573
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Anatomical Projections of Posterior and Tectal Commissures of the Pigeon

**Previous Serial Number:** None

**Principal Investigator:** Dr. Laurence J Stettner

**Other Investigators:** Dr. Ronald E. Myers

**Cooperating Units:** None

**Man Years:**

| | |
|---|---|
| Total: | .55 |
| Professional: | .50 |
| Other: | .05 |

**Project Description:**

Objectives: To describe the connections of the posterior and tectal commissures of the pigeon, with a view toward evaluating their role in inter-ocular transfer of information.

Methods Employed: Several pigeons will be operated in order to sever the posterior and tectal commissures. Following this, after survival times varying between 8 days and 21 days, the birds will be sacrificed and perfused. The brains are then embedded and stained for degenerating axons with modified Nauta technique, alternate sections being stained for Nissl.

Major Findings: In the course of surgery, a much improved anesthetic technique has been developed for birds. It involves respiration with a mixture of fluothane and oxygen. Anatomical results are just now coming in. Preliminary analysis indicates major projections to the deeper layers of the tecta, supporting the supposition of a prominent role for these structures in transfer of visual information.

Significance: Birds have complete cross over in the optic chiasm of fibers coming from the retina. They have a very primitive cortex, and no corpus callosum. The structures involved in inter-ocular transfer have not been identified. Anatomical specification of the connections of the commissures in the avian brain is an important step towards

further understanding of its functional organization.

Proposed Course of the Project: To continue with the project as described above.

Publications: None

Serial No. NDB(I) 68-LPP 1574
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Survey of infertility in a caged colony of Macaca Mulatta

Previous Serial Number: None

Principal Investigator: Dr. Thomas O. McCann

Other Investigators: Dr. Ronald F. DiGiacomo

Cooperating Units: Columbia Presbyterian Medical Center, New York, N.Y.

Man Years:

| | |
|---|---|
| Total: | 0.58 |
| Professional: | 0.50 |
| Other: | 0.08 |

Project Description:

Objectives: To set up clinical criteria for the determination of infertility, to study the reproductive organs of females meeting this criteria and finally to correlate the clinical and pathological findings in order to determine causes of infertility in the monkey.

Methods Employed: Once the clinical criteria were determined, a group of females from the breeding program meeting this criteria were sacrificed and their reproductive organs studied. Another group, released from the breeding program for other reasons, constituted a comparison group.

Major Findings: Two criteria of clinical infertility evolved: (1) Eleven successive matings without conception or (2) very irregular menses with long periods of amenorrhea. This group of females was further divisible into primary infertile (those never conceiving in the Laboratory) and secondary infertile (those conceiving in the Laboratory but since becoming infertile). The primary infertile group were characterized by a high incidence of irregular menses with menorrhagia and periods of amenorrhea. The secondary infertile group also experienced menorrhagia and amenorrhea but to a lesser extent. The percentage of pathological findings in all three groups primary infertile, secondary infertile and control - was approximately the same - 65%. Pathological findings included endometriosis, leiomyomata uteri, ovarian cysts and endometrial polyps.

The findings of endometriosis will form the basis of another study. Although pathological findings were many - it was felt that the changes were not of sufficient magnitude to cause infertility and hence only a few females were classed as infertile for pathological reasons. Two other factors were also thought to play a role in infertility - (1) endocrine abnormalities and (2) behavior factors.

Significance: This project is part of a large program to increase the efficiency of the breeding program, the information on infertility and its causes adds greatly to our understanding of reproductive problems.

Proposed Course of the Project: Project has been completed.

Publications: None.

Serial No. NDB(I) 68-LPP 1575
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

**Project Title:** Comparative Studies of the Telencephalon

**Previous Serial Number:** None

**Principal Investigator:** Dr. Sven O. E. Ebbesson

**Other Investigators:** Dr. Lennart Heimer
Dr. Theodore Voneida
Dr. Frank Scalia
Dr. Boyd Campbell

**Cooperating Units:** Department of Psychology, M.I.T.
Department of Anatomy, Case Western Reserve University
Department of Anatomy, Downstate Medical Center, New York
Center for Neural Sciences, Indiana University

**Man Years:**
Total .4
Professional: .2
Other: .2

**Project Description:**

Objectives: 1) To define patterns of central nervous system organization in primitive vertebrates. 2) To make a survey of the afferent and efferent fiber systems of the telencephalon in several species from each vertebrate class.

Methods Employed: Lesions of the olfactory tracts and in various portions of the telencephalon have been made in the Tegu lizard, the moray eel, and nurse shark. The animals are sacrificed 5 to 40 days after surgery and the brains are then processed according to several modifications of the Nauta method. The silver impregnated degenerated axons are identified and reconstructions are made of the course and termination of the pathways under investigation.

Major Findings: 1) The olfactory tract was sectioned unilaterally in four nurse sharks and one tiger shark and the animals were killed between 6 and 43 days after the operation. Whereas the axon degeneration

was demonstrated best with the Nauta-Laidlaw technique after 3-4 weeks survival time, the terminal degeneration, as identified in Fink-Heimer preparations, was only seen in a 14-day specimen. The distribution of olfactory tract fibers is entirely ipsilateral to the lesion, the majority terminating near the olfactory peduncle. Profuse terminal degeneration is observed in a nucleus at the base of the olfactory peduncle and in the lateral olfactory area (Johnston '11). Some terminal degeneration is also seen rostrally, near the surface, in the dorsolateral quadrant of the hemisphere. Caudally the distribution of terminal degeneration in the lateral olfactory area gradually shifts ventrally and medially and extends as far as the caudal portion of the area superficiale basalis (Johnston '11), where some degenerating fibers reach the midline.

2) In order to study the pallial commissural systems in the lizard, unilateral lesions of various sizes were made in telencephalic structures of 23 animals. Hemispherectomy cases revealed that fiber degeneration in the cortical mantle was confined to the medial half. The piriform cortex, pallial thickening, and general cortex (Curwen '37) were free of degeneration. Terminal degeneration was observed in the following areas: 1) among the dorsal dendrites of the neurons in the medial half of the dorsomedial cortex. 2) around the perikarya and dendrites of the dorsal part of the hippocampal cortex. 3) in the ventral cell plate of Unger ('06), and 4) in the dorsomedial part of the hippocampus.

Significance: The Nauta method provides us for the first time with a satisfactory tool for the tracing of thinly myelinated and nonmyelinated axons, and since primitive vertebrates have a great proportion of such fibers, we are now able to trace pathways that older methods failed to elucidate. The studies reported on here are the first of their kind and several more species from each vertebrate class must be examined before the significance of these observations can be discussed.

Proposed Course of the Project: To define the cytoarchitecture and connections of the telencephalon in representative species of fish, amphibians, and reptiles, using the methods of Nissl, Golgi, and Nauta.

Publications: None.

Serial No. NDB(I) 68-LPP 1576
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Comparative Studies of the Mesencephalon

Previous Serial Number: None

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Dr. Kalman Rubinson
Dr. Harvey Karten
Dr. James M. Petras

Cooperating Units: Department of Psychology, M.I.T.
Department of Neurophysiology, Walter Reed Army Institute of Research

Man Years:
Total: 1.0
Professional: .7
Other: .3

Project Description:

Objectives: 1) To define patterns of central nervous system organization in vertebrates. 2) To make a survey of afferent and efferent connections of the mesencephalon in several species from each vertebrate class.

Methods Employed: The animals are sacrificed 5-30 days after lesions are made and the brains are then processed according to the method of Nauta. The silver impregnated degenerating axons and terminals are identified and reconstructions are made of the course and termination of the pathways.

Major Findings: The projections of the optic tectum have been worked up in detail for the frog and lizard. Following deep lesions of the caudal portion of the optic tectum of the frog, degeneration was traced with a modified Nauta technique to the contralateral tectal hemisphere via the tectal commissure, and to the pretectum and ipsilateral neuropil of Bellonci via a medial ascending tract. Other ascending pathways pass medial to the lateral marginal optic tract and also within the commissura transversa to reach ipsilateral and contralateral thalamic nuclei, the suprachiasmatic hypothalamic region, and

the contralateral mesencephalic tegmentum. Descending tracts project, ipsilaterally, to reticular formation, interpeduncular nucleus, nucleus isthmi, and superior olive. Contralateral projections were to the reticular formation and the medial intermediate region of the cervical spinal cord.

More rostral lesions showed the same pattern with the addition of degeneration in the motor root of the trigeminus, the region ventrolateral to the trigeminal motor nucleus, and the descending trigeminal tract. These additional projections are interpreted as those of the trigeminal mesencephalic nucleus. The projections of the tectum in the lizard, are remarkably similar although the accepted nomenclature does not always agree, e. g., the projection to the neuropil of Bellonci in the frog is probably homologous to the projection to the ventral portion of the lateral geniculate in the lizard.

Significance: Comparative studies such as these are carried out in attempts to elucidate problems of human central nervous system organization by broadening our understanding of brain organization of lower vertebrate forms. These studies are the first comprehensive studies of their kind using the Nauta method. The findings in the frog and lizard are, with few exceptions, similar to those observations made in mammals. A larger sample of species must be examined before the significance of these observations can be ascertained.

Proposed Course of the Project: 1) To examine representative species of fish, amphibians, and reptiles for variation in afferent and efferent pathways of the mesencephalon. 2) To define in these species the cytoarchitecture of the region using Nissl and Golgi preparations.

Publications: None.

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